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Dr S.R.Padwal-Desai has taken over charge as President of the Association of Food Scientists & Technologists (India) on 24 November 2000 at a brief ceremony held at the end of ICFOST-2000.

Dr Padwal-Desai, M.Sc., M.S., Ph.D., FAFST, FAAS had a distinguished scientific career at the Bhabha Atomic Research Centre (BARC), Mumbai, before his retirement as Head, Food Microbiology Group of the Food Technology Division. His pioneering work at BARC included Radiation Preservation of Foods such as potato, onion, fruit, fish and spices. He has made great efforts through electronic media, radio and seminars to popularize food technology.

Dr Padwal-Desai was responsible for establishing a very active research group for control of post-harvest pathogens in fruits, vegetables, cereals and pulses. He has organized several symposia, conferences, and workshops on food irradiation, quality assurance and product development. He is an advisor on food for International Atomic Energy Agency,Vienna.

Dr Padwal-Desai has guided a number of students for M.Sc., Ph.D. and is a visiting faculty at the University Department of Chemical Technology, SNDT Women's University and Mumbai University.

Dr Padwal-Desai has been closely associated with many professional bodies like Association of Food Scientists & Technologists (India), AMI, ISEST, INS, EMSI, NAARRI, IIChemE, LSI, and held key positions in these organizations. He was also responsible for creating a rapport between AFST(I) at Mumbai and food industry, academia and professional organizations. In recognition of his services to promote Food Science and Technology, Dr Padwal-Desai was elected Fellow, Association of Food Scientists & Technologists (India). He was also conferred the Fellowship of the National Academy of Agricultural Sciences in 1999. Dr Padwal-Desai is closely involved with organizations working towards the industrial developments in Konkan, Goa and Kutch areas.

On behalf of the editorial board of **Journal of Food Science and Technology**, I look forward to a closer interaction with President and his team and wish him all success during his tenure.

B.R. Lokesh
Editor-in-Chief

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JOURNAL OF FOOD SCIENCE AND TECHNOLOGY

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RESEARCH PAPER

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Effect of Physico-chemical Conditions and Yeast Enrichment on *Punjabi warri* Fermentation

S.K. SONI[1]*, D.K. SANDHU[2] AND RAMAN SONI[3]

[1]Department of Microbiology, Panjab University, Chandigarh-160014, India.
[2]Department of Microbiology, Guru Nanak Dev University, Amritsar-143005, India.
[3]Department of Microbiology, Kanya Maha Vidyalaya, Jalandhar-144001, India.

An improvement in the nutritional and acceptable sensory characteristics of traditional *Punjabi warri* fermentation was achieved through a combination of physico-chemical factors namely, incubation of *warri* doughs at 28°C with an initial pH of 4.5 and supplementation with 1-2% sucrose. Enrichment of dough with *Saccharomyces cerevisiae* in combination with the native bacterial flora of the ingredients was found to be the best microbial environment for optimum *warri* fermentation.

Keywords : *Punjabi warri,* Fermentation, Optimization, Physico-chemical conditions, Yeast enrichment.

India has innumerable sects of human population and the diversity of food habits has resulted in a large number of traditional fermented foods. Many of these remain highly secretive in their preparation being passed on from generation to generation and tend to be regionalized with many of them being made only on home scale (Sankaran 1998; Soni and Sandhu 1999). *Warris,* somewhat like Japanese *miso* (Batra 1981), belong to a very famous group of legume-based fermented foods of Northern India. These are spicy, hollow, brittle, friable balls, very popular in Amritsar district of Punjab State and used as a condiment in cooking with vegetables, legumes or rice. These are manufactured according to the traditional, technologically less advanced methods, using simple equipment and are produced on cottage industry scale, using natural microflora from the staples and the surroundings. As a result, the ultimate products may have undesirable attributes. Several bacterial organisms like *Leuconostoc mesenteroides* and *Streptococcus faecalis* and yeasts-*Saccharomyces cerevisiae, Pichia membranaefaciens, Kluyveromyces marxianus* and *Candida krusei* occurring as the native flora in the ingredients are known to bring about various changes such as acidification and leavening besides improving the digestibility and nutritional value of dough in traditional *punjabi warri* fermentation (Sandhu et al. 1986; Sandhu and Soni 1989 ; Soni and Sandhu 1990a, 1999; Sankaran 1998). Certain important biochemical changes include increases in soluble solids, non-protein nitrogen, soluble nitrogen, free amino acids, proteinases and water soluble vitamins like B_1, B_2 and B_{12} (Soni and Sandhu 1990 a, 1999, Sankaran 1998)

Since the traditional *warri* fermentations are carried out at atmospheric temperature and the seasonal changes affect microbial types (Sandhu and Soni 1989; Soni and Sandhu 1990a), there arises a need for standardizing various physico-chemical factors and the microbial inocula for further improving the nutritional and organoleptic properties of the product. The present investigation was undertaken to study the effect of temperature, pH, supplementation of some free sugars in the fermenting ingredients and different microbial combinations to optimize *warri* fermentation.

* *Corresponding Author*

Materials and Methods

The traditional method of preparing *warri* doughs (control) was on the lines as described earlier (Sandhu and Soni 1989; Soni and Sandhu 1990b). This involved the grinding of blackgram (*Phaseolus mungo*) grains to a paste after overnight soaking. The paste was then added with asafoetida (*Ferula foetida,* 0.5-1.0%), caraway (*Carum carvi,* 0.5-1.0%), cardamom (*Elettaria cardamomum,* 1.0%), cloves *(Syzygium aromaticum,* 0.4-0.5%) fenugreek *Trigonella faenumgraecum* 1.0%), ginger (*Zingiber officinale,* 8.0%) and red pepper (*Capsicum annum,* 1.0%). The resulting paste/dough was then moulded into balls of 3-5 cm diameter and allowed for simultaneous fermentation and drying in open air for 4-8 days, unless otherwise stated.

Optimization studies : The studies on the effects of various physico-chemical factors, keeping in view the optimum values for bacteria and yeasts were as follows :

Effect of temperature on *warri* fermentation was studied by fermenting the sets of traditionally prepared doughs at 28°C and 40°C, respectively, with initial pH between 5.5-6.0, by incubating in the temperature controlled rooms.

Effect of pH on *warri* fermentation was studied by adjusting the initial pH of the doughs to 4.5 and 7.5, respectively, with 1% citric acid and 1% sodium citrate and fermenting at room temperature (30-35°C).

Effect of free sugar was studied by supplementing the traditionally prepared doughs with sucrose at levels of 1 and 2%, respectively and fermenting at room temperature of 30-35°C and initial pH of 5.5. The dough without any supplemented sugar was taken as control.

Effect of yeast enrichment was studied by inoculating different sets of doughs, separately, with four yeast strains-*Saccharomyces cerevisiae, Pichia membranaefaciens, Kluyveromyces marxianus* and *Candida krusei* without eliminating the natural microflora of the ingredients, which comprised mainly of *Leuconostoc mesenteroides* and lactobacilli (Soni and Sandhu 1990a). This was done by taking a suspension of 48 h old yeast culture in 5 ml sterile water and mixing into each batch of dough, comprising 200 g dry ingredients. Un-inoculated dough was taken as control.

Microbiological and biochemical analysis : The pattern of total microbial load and the levels of other biochemical and nutritional constituents attained in different fermentation sets were analysed successively at 24 h intervals from zero hour till the end of fermentation (5 days). Total bacterial and yeast counts were determined in the samples after making the appropriate dilutions and pour plating, using the appropriate media (Sandhu and Soni 1989). For biochemical analysis, 10 g samples were blended with 90 ml distilled water for few minutes. An aliquot of 20 ml was centrifuged for 10 min (8500 at 4°C) and the supernatant was analyzed for various soluble parameters. pH was noted directly using Beckman Zeromatic pH meter. Soluble solids (AOAC 1975), reducing sugars (Miller 1959), soluble proteins (Lowry et al. 1951), amylase activity (Bernfeld 1955) and proteinase activity (Makonnen and Porath 1968), free amino acids (Troll and Cannon 1953), vitamin B_1 (Gupta and Codwallander 1968), vitamin B_2 (AOAC 1975) were assayed. Total nitrogen, non-protein nitrogen and total proteins (AOAC 1975) were, however, estimated directly from the dough samples. *Warris* prepared from all the doughs were also evaluated organoleptically in terms of appearance, taste, flavour and overall acceptability by a panel of 6 judges using a 9-point Hedonic scale (Swaminathan 1979).

Results and Discussion

The pattern of changes occurring in different sets of *warri* doughs, fermented under varying physico-chemical conditions, is represented in Fig 1-3. Incubation at 40°C favoured the rapid multiplication of bacterial population, which produced higher acid and gas, indicated by a sharp decline in pH and rise in volume. This temperature also proved to be optimum for the proteolytic organisms, responsible for increased production of proteinase, which probably brought about the degradation of higher proteins as revealed by the lower levels of total protein content in the doughs fermented at 40°C. However, the lower levels of soluble nitrogen and free amino acids at this temperature were probably due to incomplete breakdown of proteins and/or due to their immediate utilization by the rapidly increasing bacterial population. On the other hand, a temperature of 28°C favoured the rapid propagation and activity of yeasts, which probably contributed SCP, amylases, amino acids and vitamins B_1 and B_2 as indicated by significant rises in these constituents along with soluble solids (Fig. 1). The yeasts isolated from Indian fermented foods have already shown their ability to assimilate starch by producing amylases and to grow in vitamin-free medium (Soni and Sandhu 1990a, 1999). This temperature also resulted in the product with better appearance, taste, flavour and overall acceptability (Table 1), which might be due to the right levels of leavening, acidification and the aroma yielding compounds produced by higher yeast population.

Initial pH around 7.5 favoured the occurrence and rapid multiplication of bacterial population, thus accounting for the higher bacterial counts in the respective doughs (Fig. 2), which produced a higher degree of acidification and leavening as indicated by a sharp decline in pH and increase in volume. On the other hand, initial acidic pH (4.5) favoured the occurrence, growth and prevalence of yeast population, which probably contributed SCP and vitamins B_1 and B_2 as revealed by the increased levels of these in the respective doughs. This acidic pH was probably optimum for the production and activities of amylases and proteinases, thus liberating higher levels of soluble solids, free amino acids, non-protein nitrogen and soluble nitrogen (Fig.2). The product obtained with this dough was also better organoleptically in terms of taste, flavour and overall acceptability (Table 1), which might be due to the optimal leavening, acidification and the aroma yielding compounds produced at this pH.

Supplementation of free sugar supported the multiplication of both the bacteria and yeasts, as it acted as a simple energy source for the microbes, which remained viable and active for a longer period as evident from the higher microbial load in these batches as compared to control. The addition of sucrose also brought about pronounced increases in various nutritional constituents including reducing sugars, soluble solids, free amino acids and vitamins B_1 and B_2 besides acidification and leavening (Fig. 3). The higher levels of the reducing sugars in the sucrose supplemented batches might be due to invertase formation by the microbes and the subsequent breakdown of sucrose. Proteinase levels (acidic; pH 5.0), though, were identical in all the sets, the differences in non-protein nitrogen, soluble nitrogen, total proteins, soluble proteins and free amino acids were perhaps due to the induction of other proteases (neutral and alkaline) by the sucrose, which probably resulted in the degradation of proteins including the constitutively produced enzymes *in vivo* conditions. The increased vitamin B_1 and B_2 levels were perhaps due to the presence of yeasts being a good source of these vitamins. The products made from such doughs got the better scores for their organoleptic properties

TABLE 1. SENSORY SCORES OF *WARRIS* PREPARED FROM DOUGHS FERMENTED UNDER VARIABLE PHYSICO-CHEMICAL AND MICROBIAL PARAMETERS

Warris made from doughs obtained from different sets	Appearance	Taste	Flavour	Overall acceptability
Effect of temperature				
28°C	8.2	8.0	8.3	8.3
40°C	8.0	7.8	7.6	8.0
Effect of initial pH				
4.5	8.2	8.0	8.1	8.4
7.5	7.9	7.9	7.8	8.0
Effect of sugar supplementation				
1%	8.2	7.9	8.3	8.4
2%	8.2	7.9	8.2	8.4
None	7.9	7.6	7.6	7.9
Effect of microbial domination				
Natural flora +*S. cerevisiae*	8.3	7.9	8.4	8.5
Natural flora + *P. membranaefaciens*	8.0	7.9	8.1	8.1
Natural flora + *K. marxianus*	7.9	7.8	8.2	8.0
Natural flora + *C. krusei*	8.1	7.9	8.0	8.1
Natural flora only	8.0	7.8	7.9	7.9

Values are mean of scores given by a panel of 6 judges on a 9-point Hedonic scale

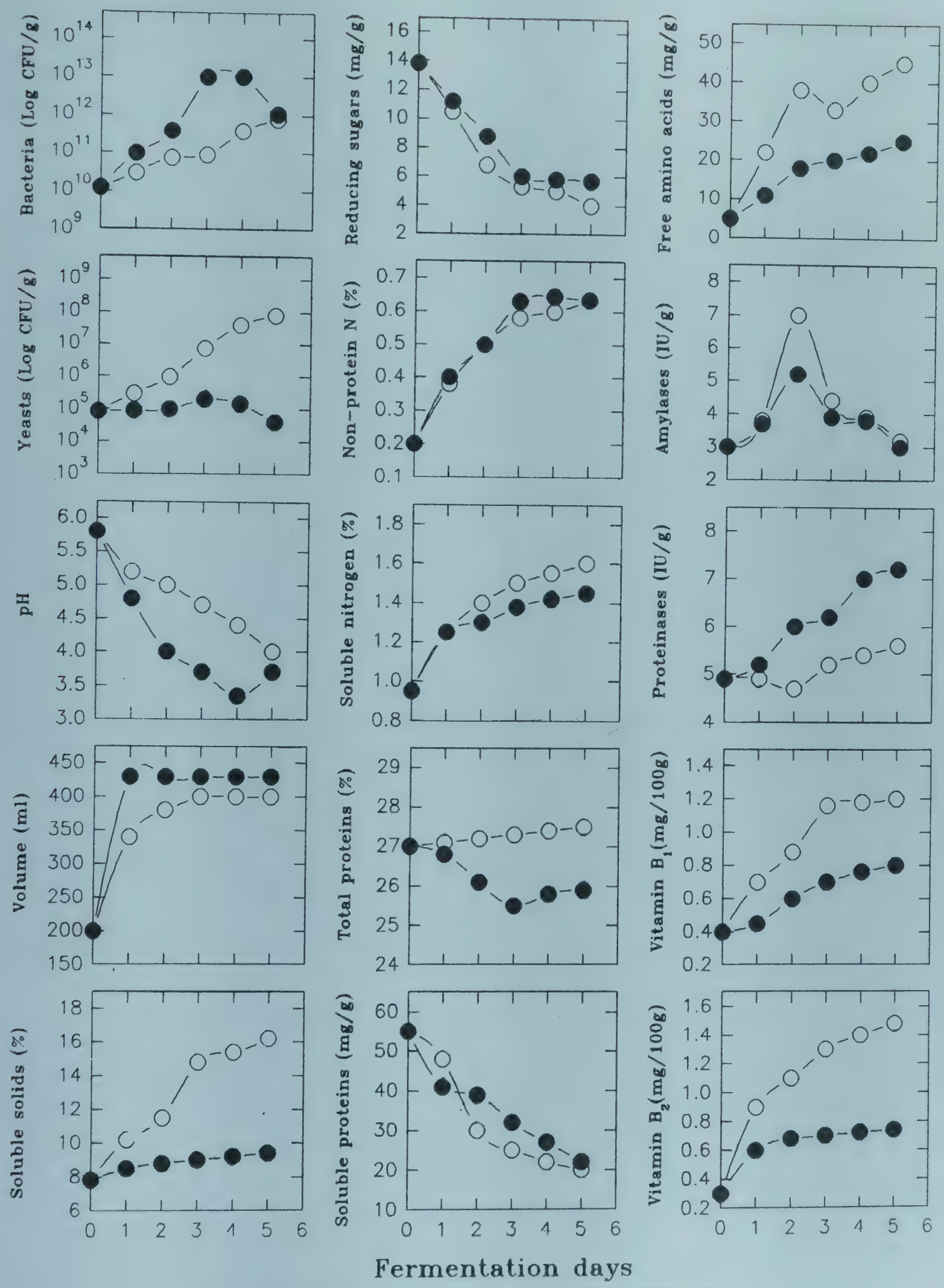

Fig. 1. Effect of temperature on *warri* dough fermentation

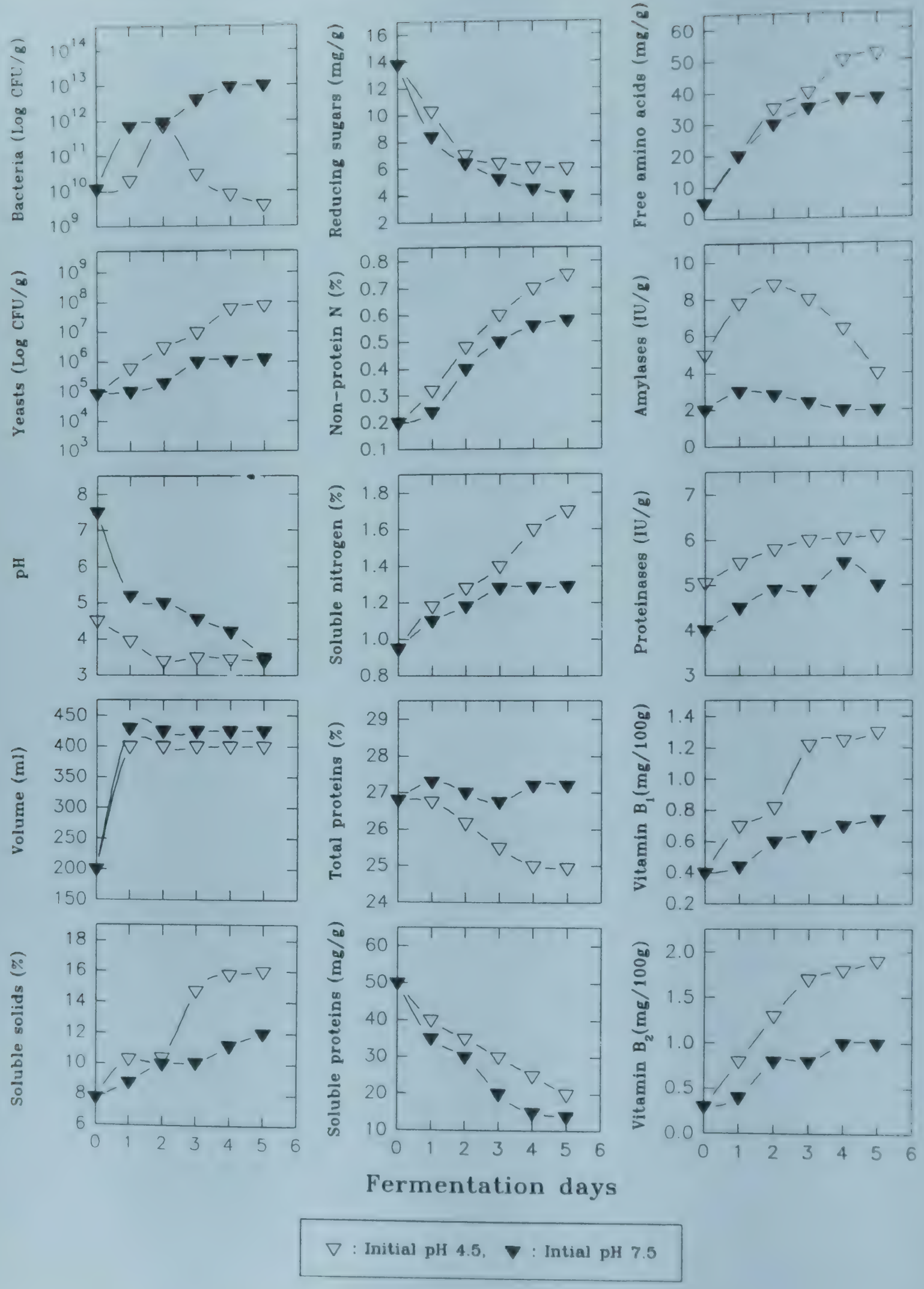

Fig. 2. Effect of initial pH on *warri* dough fermentation

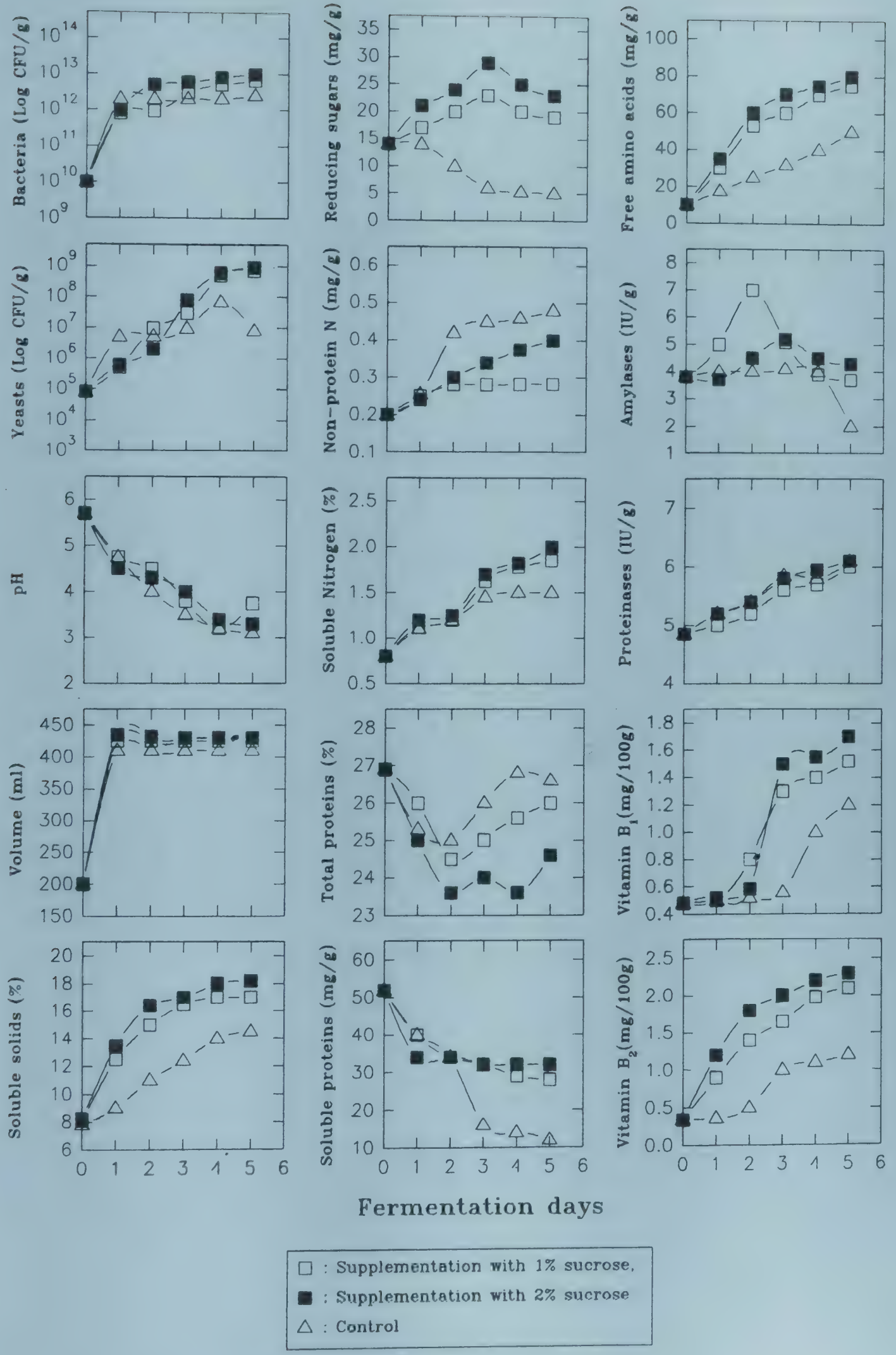

Fig. 3. Effect of sugar supplementation on *warri* dough fermentation

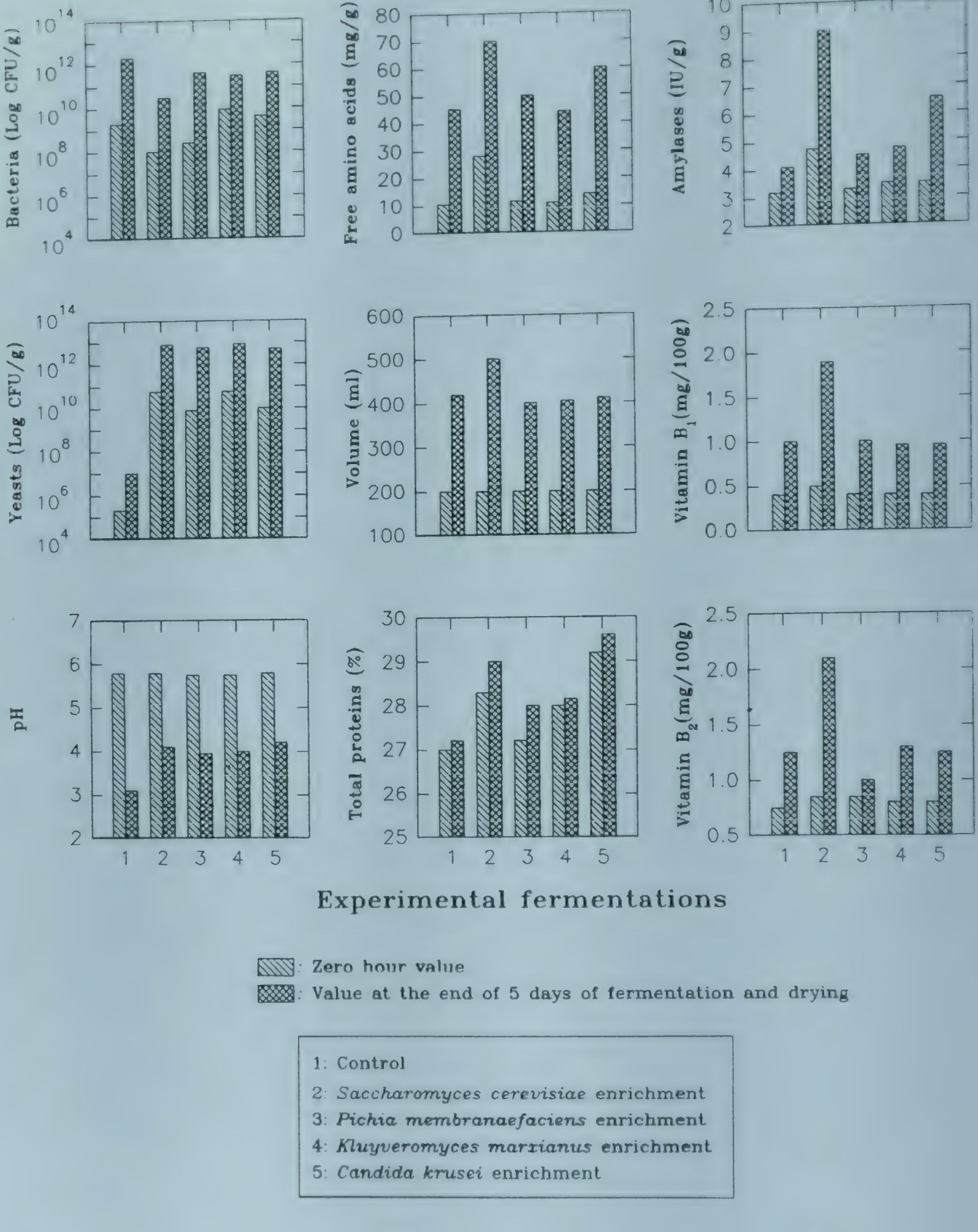

Fig. 4. Effect of yeast enrichment on *warri* dough fermentation

due to the optimal activities of the desired organisms, for the synthesis of aroma yielding compounds, as compared to the control, which was not supplemented with any sugar (Table 1). Further, the levels of these constituents attained in the presence of 2% sucrose were comparatively higher than in the case of 1% sugar. All these observations corroborate the earlier observations on *dosa* and *idli*, other indigenous fermented foods of India (Sandhu and Soni 1988; Soni and Sandhu 1989 a, b; Soni and Sandhu 1991).

The comparison of the mean values of various constituents attained in bacterial dominated (control) and yeast dominated fermentation sets is shown in Fig. 4. *Saccharomyces cerevisiae* enriched fermentations were found to be the best in terms of leavening and final levels of amylases, free amino acids, total proteins as well as vitamins B_1 and B_2. *Candida krusei* enriched batches also exhibited good amylolytic activity, free amino acids and total proteins. The remaining two experimental fermentations did not exhibit significant differences in their nutritional levels as compared to control. Biochemical characterization of the predominant microorganisms involved in *warri* fermentation (Sandhu and Soni 1989; Soni and Sandhu 1990a) confirmed the production of amylases and vitamins by yeasts used in the enrichment as indicated by their ability to assimilate starch and grow in vitamin-free medium. The increased levels of total proteins and free amino acids were probably due to the domi-nation of yeast population, which is a better source of SCP and amino acids. Further, *S. cerevisiae* also added to leavening as a result of its ability to ferment several carbohydrates. This organism probably also produced several aroma producing compounds as indicated by the most acceptable sensory characteristics observed in its respective set (Table 1). Most of the yeasts also contributed single cell protein (SCP) as indicated by high protein contents observed in their respective batches. Samples of *warris* made from yeast dominated doughs got the better sensory scores as compared to the product made from bacterial dominated control (Table 1). Maximum acidification as found in bacterial dominated control was perhaps due to the ability of *Leuconostoc mesenteroides* and lactobacilli, the principal bacteria associated with natural fermentation (Sandhu et al. 1986; Soni and Sandhu 1990a; 1999). The role of *S. cerevisiae* in further improving the digestibility, amylolytic activity and nutritive value of *dosa* and *idli*, other Indian fermented foods, has also been reported earlier (Soni and Sandhu 1990a, 1991, 1999).

It is apparent that standardization of indigenous *warri* fermentation with respect to incubation temperature, initial pH, supplementation of free sugar and *Saccharomyces cerevisiae* enrichment, in combination with the native bacterial flora, can play a significant role in improving the nutritional quality and acceptability of the product.

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RESEARCH PAPER

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Storage Stability of Colour Substance in Thermally Processed Dry Chilli Powder

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Changes in carotenoid pigments as well as ascorbic acid content of dry chilli powder during various conditions of storage were investigated. Ascorbic acid when added at 10% level into the thermally processed chilli powder substantially reduced colour impairment during storage. Losses of carotenes to the extent of 13.47-36.62% were observed in the treated chilli powder after 150 days of storage at 40°C/70% RH and 30°C/90% RH, respectively. The unprocessed samples were heavily contaminated with bacteria and fungi compared to the processed and treated samples.

Keywords : Spices, Chilli, Thermal processing, Storage, Ascorbic acid, Carotenes.

Chilli is cultivated in all the States and Union Territories of the country. India is the highest chilli exporting country. A common problem faced by both importing and exporting countries is that spices are often contaminated with micro-organisms, which are the major sources of contamination in the prepared food products. Besides, various sensory qualities i.e., colour and flavour are also lost during transportation by sea or delay in clearance of the containers from the port of entry. Bulk of chilli powder gets exposed to adverse storage conditions, which favour deterioration in quality. Colouring compound of dry chilli is due to its carotene content (Pruthi 1976). Dry chillis also contain fair amounts of ascorbic acid (Jaiswal 1986). The role of ascorbic acid as an antioxidant in the prepared food products is well established (Chauhan et al. 1998). The present investigation was undertaken to study the effect of ascorbic acid on the storage stability and colour of thermally processed and raw dry chilli powder.

Materials and Methods

Dry red chilli fruits (*Capsicum annum*) were obtained from the Department of Vegetables, JNKVV, Jabalpur, India. Chilli fruits were ground using water jacketted electrical grinder fabricated for the purpose in the department. Ground powder was sieved to pass through a 60 mesh (B.S.) sieve. The moisture content was determined by AOAC (1984) methods. The carotene content was determined by following the method of Winton and Winton (1999). Direct colorimetric method (Ranganna 1979) was used to estimate ascorbic acid content. Total plate counts were determined using AOAC (1975) method. The fungi were identified using standard methods (Sutton 1980, Thom and Rapper 1985). All estimations were carried out in triplicate and an average data of these are reported.

Thermal processing of chilli powder : Chilli powder (100 g) was packed in glass jars of 4"×12.5" size. The mouth of the jar was plugged with cotton and then closed wrapping with aluminium foil. The packed glass jars were autoclaved at three levels of temperature (105, 110 and 115 ± 2°C) and four levels of autoclaving time (5, 10, 15 and 20 min).

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Storage studies : Adsorption isotherm of chilli powder at 30°C was obtained by following the method of Bera et al (1990). Low density polyethylene (150 gauge) pouches (size 8 × 8 cm) were used for packaging 10 g of processed, unprocessed and treated powder (Treated samples refers to the processed sample in which ascorbic acid was added to offset the loss during thermal processing and further supplemented with 10% ascorbic acid). These packed samples were stored separately in desiccators, maintained at 30°C and 95% RH and 45°C and 70°/RH. The relative humidity inside the desiccators was maintained by sulphuric acid solutions (Ruegg 1980). These desiccators were placed inside the BOD incubator, where pre-determined temperature was maintained. The pouches were weighed every week and changes in any physical parameter i.e. colour, caking and microbial growth were recorded. The shelf life of packed product was estimated by determining the critical moisture content of the product from the following equations :

$$\text{In } Mc\text{-}M/Me\text{-}Mo = -kt \quad \text{.....(1)}$$

where, Me = equlibrium moisture content, Mc = critical moisture content, Mo = initial moisture content and t = storage time.

An analytical method has been derived for the determination of critical moisture content (Mc) by using Henderson's equation (1952) as follows :

$$(1\text{-}a_w) = \bar{e}^{KTM^n} \quad \text{.....(2)}$$

$$\log (1\text{-}a_w) = -KTM^n$$

$$\log \log [1/1\text{-}a_w) = n\log M + \log K + \log T$$

$$M^n = -\log (1\text{-}a_w/_{KT})$$

$$M = [-\log (1\text{-}a_w)/_{KT}]^{1/n}$$

$$M = [1/_{KT}]^{1/n} \quad [\log 1/1\text{-}a_w)^{1/n} \quad \text{.....(3)}$$

Now, differentiating for M in the equation with respect to a_w, the following was obtained.

$$dM/da_w = [1/_{KT}]^{1/n} \; 1/n[\log 1/1\text{-}a_w]^{1/n}[-(1\text{-}a_w)] \quad \text{.....(4)}$$

Equating the equ (4) to zero and solving for a_w would give a_w = 1 when "M" is maximum. Turning point of dM/da_w versus a_w was found by simultaneously differentiating dM/da_w with respect to a_w and equating it to zero and then again solving for a_w gave the critical relative humidity (ac), as given below:

$$\ln (1-ac) + (1/n-1) (1-ac)^2 = 0 \quad \text{.....(5)}$$

$$\ln X + (1/n-1) X^2 = 0 \quad \text{.....(6)}$$

where 1-ac=X and n=Henderson equation constant. The value 'N' is substituted in the equ. (6) and the value of 'x' could be calculated by Newton's method of iteration. From this critical relative humidity, corresponding critical moisture content (Mc) could be obtained from the EMC-relative humidity graph.

Results and Discussion

The initial moisture content, ascorbic acid and carotene contents of chilli powder were 9.5% (d.b), 53.33 mg and 0.7545 mg/100 g, respectively. The initial microbial load of chilli powder was 85 × 10 cfu/g. Chilli powder contained 20 × 10 cfu/g bacteria and 63 × 10 cfu/g fungi. Species of *Penicillium, Aspergillus, Rhizopus, Fusarium* and *Trichoderma* were identified. Chilli powder thermally processed at 105, 110, 115°C±2°C for 5, 10 and 15 min, respectively did not show any microbial growth both in the nutrient agar and potato dextrose agar. Therefore, it was inferred that sterilization of chilli powder could be achieved at a lesser autoclave temperature (105°C) and time (5 min.).

Effect on the processing temperature and time on ascorbic and carotene was not much pronounced. However, 17-20% losses in ascorbic acid and 10-12% losses in carotene contents were observed in the processed chilli powder, irrespective of their processing time and temperature. Therefore, further studies on storage was conducted with the chilli powder, processed at 105°C and 5 min. of autoclaving time.

Adsorption isotherm of chilli powder : It was observed that isotherm at 30°C was of sigmoid shape (type II). The rate of moisture adsorption was slow or less below the water activity (a_w) of 0.5, but thereafter it increased rapidly showing a steep rise in moisture content (Fig. 1). At a water activity of 0.9, the processed chilli powder had 0.184 kg water/kg dry solid. The rate of moisture adsorption of processed chilli powder was more or less same as that of unprocessed powder (Selot 1989). Henderson's equation (2) was fitted adequately (r = 0.864) to the isotherm data and a working form of an equation ($1-a_w=e^{3.644}M^{0.25}$) was formed. The value of n = 0.25 was further used for the calculation of critical relative humidity from the equation (6). Critical relative humidity of chilli powder at 30°C was 53%, which corresponded to the equilibrium moisture content 11.4% (d.b). Therefore, critical moisture content (Mc = 11.4%) of chilli powder was used in the equation (1) to predict shelf life period during storage.

Storage characteristics of packed chilli powder : The pattern of moisture adsorption of treated and control samples stored at 30°C/90% RH and 40°C/70% RH for 150 days were more or less same. However, it has been observed that treated samples adsorbed slightly more moisture (12.50% d.b) than the unprocessed samples (11.75% d.b). The changes in the ascorbic acid and carotene content are given in Table 1. At a temperature 40°C and 70% RH, the losses in the ascorbic acid and carotene contents were less in unprocessed samples in comparison to the samples, stored at 30°C and 90% RH. The losses in the ascorbic acid in the processed, treated and unprocessed samples stored at 40°C and 70 % RH were 28.12, 18.67 and 21.69%, respectively (Table 1). The carotene contents of the samples decreased from 0.745 (initial) to 0.501,0.570, 0.520 mg/100 g in unprocessed, treated and processed samples, respectively when stored at 40°C and 70% RH.

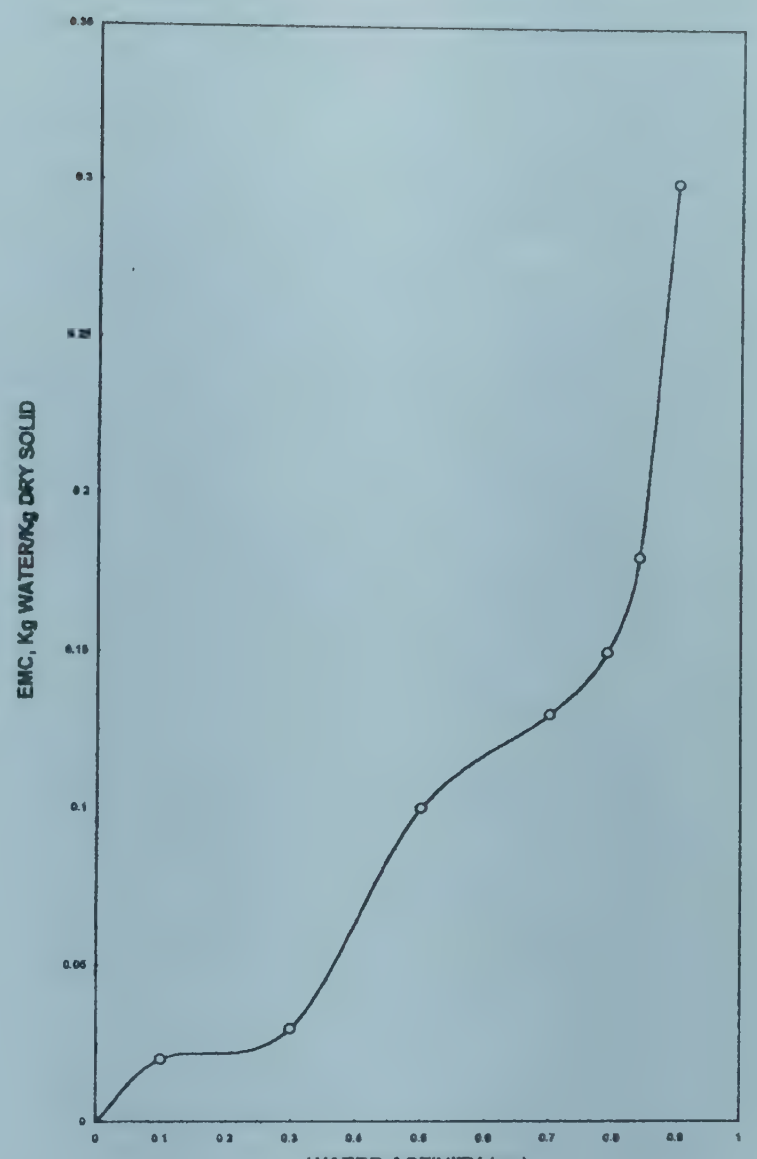

Fig. 1. Moisture adsorption isotherm of processed red chilli powder at 30°C

TABLE 1. EFFECTS OF STORAGE TEMPERATURE AND RELATIVE HUMIDITIES ON ASCORBIC ACID AND CAROTENE CONTENTS OF CHILLI POWDER

Storage conditions	Ascorbic acid content, mg/100g			Carotene content, mg/100g		
	Initial	After storage	Loss, %	Initial	After storage	Loss, %
30°C,90%RH						
A	53.33	30.00	43.25	0.74	0.35	43.55
B	67.72	55.07	18.67	0.67	0.43	36.22
C	44.26	28.66	35.52	0.67	0.38	42.36
40°C,70%RH						
A	53.33	38.00	28.12	0.74	0.501	32.75
B	67.72	55.07	18.67	0.67	0.578	13.47
C	44.26	28.66	35.52	0.67	0.520	22.15

A = Unprocessed, B = Treated, C = Processed

On visual examination of the samples stored at 30°C and 90% RH, it was observed that except treated samples, other samples were discoloured or red colour fainted. Shakunatala and Shadaksharaswamy (1995) reported that ascorbic acid was unstable especially on exposure to heat and moisture. On mild oxidation, ascorbic acid was converted into dehydro ascorbic acid. Dehydo ascorbic acid was further converted into diketogluconic acid in the presence of any weak acid. Chilli powder contains 16% fat (Selot 1989) and when stored in the high relative humidity (90%), there may be a chance of lypolysis of fat to produce free fatty acids (weak acid), which might have helped in the conversion of dehydro ascorbic acid to diketogluconic acid, which does not possess antioxidant property of ascorbic acid. Hence, it could not protect the carotene of chilli powder from oxidation and subequent loss of red colour. On the other hand, chilli powder stored at 70% RH retained the colour of chilli powder, because the loss in the ascorbic acid content was very less compared to the loss in the sample stored in the high relative humidity (90%). Biacs et al (1992) reported that α -tocopherol and carotene helped in retaining the colour of paprika powder. Colour losses of paprika powder to the extent of 27,20 and 15% were found after 150 days of ambient storage in untreated, α-tocopherol and ascorbic acid treated paprika powders. Losses of carotene content (36.62 and 13.47%) were observed in the chilli powder treated with 10% ascorbic acid after 150 days of storage at 30°C/90% RH and 40°C/70% RH, respectively.

The total plate counts of the chilli samples stored at various storage conditions are given in Table 2. The unprocessed samples were heavily contaminated with bacteria and fungi compared to the processed and treated samples. The growth of fungi and bacteria in the samples were influenced by high or low relative humidity, temperature of storage and type of samples. Colonies of fungi and bacteria were more in the samples stored at 30°C/90 % RH in comparison to the samples stored at 40°C/70% RH. *Rhizopus* and *Aspergillus* spices of fungi were present in the samples. The addition of ascorbic acid (10%) in the processed sample did not favoured the growth of microorganism, which may be due to shift in pH of chilli sample towards lower pH.

Prediction of shelf life period : Shelf life of the samples was predicted at the level of critical moisture content. The relationships between storage period (weeks) and ln (Mc-M)/(Me-Mo) of the samples at 30°C/90% RH and 40°C/70% RH are shown in the Fig. 2 and 3. respectively. The predicted shelf life periods of untreated and treated samples were 21, 34, 25 and 45 days, respectively at 30°C/90% RH and 40°C/70% RH of storage conditions. The shelf life period of chilli powder was improved when it was mixed with 10% ascorbic acid. The actual shelf life of treated sample may be little higher than the predicted shelf life, if it is predicted based on colour intensity rather than moisture adsorption.

TABLE 2. MICROBIAL PROFILE OF CHILLI POWDER STORED FOR 5 MONTHS AT DIFFERENT TEMPERATURES AND RELATIVE HUMIDITIES

Storage conditions	Standard plate counts, cfu/g	Type of fungi species
30°C, 90% RH		
A	20.4 × 10^2	*Penicillium, Fusarium, Trichoderma, Rhizopus, Aspergillus*
B	4.0 × 10^2	*Rhizopus*
C	5.3 × 10^2	*Rhizopus*
40°C/70% RH		
A	9.8 × 10^4	*Rhizopus, Aspergillus*
B	1.6 × 10^2	*Rhizopus*
C	1.8 × 10^2	*Rhizopus*

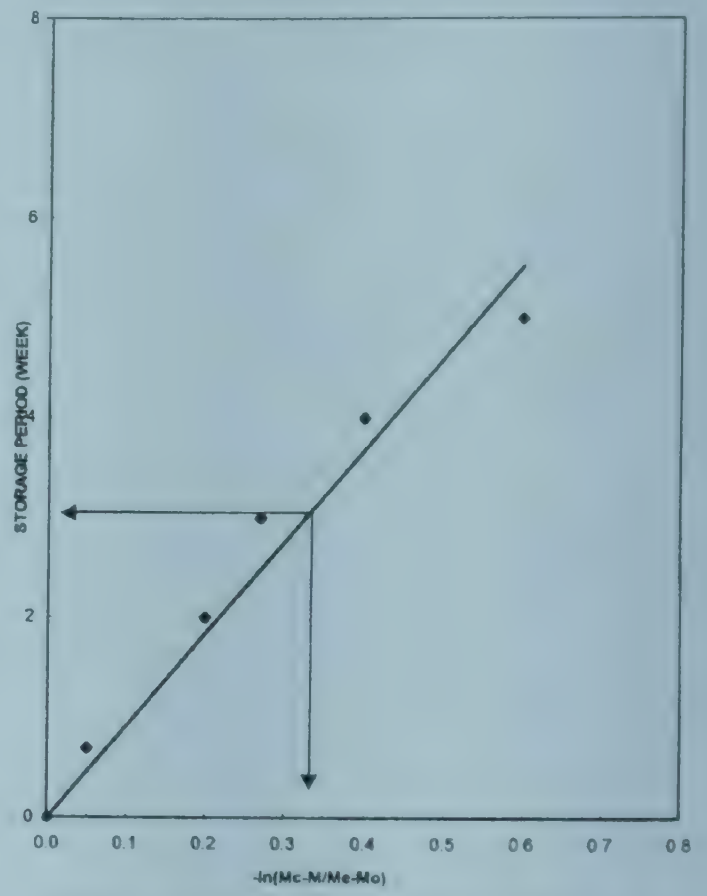

Fig. 2. Graphical representation of -ln Mc-M-M/Me-Mo and storage period of treated sample at 30°C and 90% RH

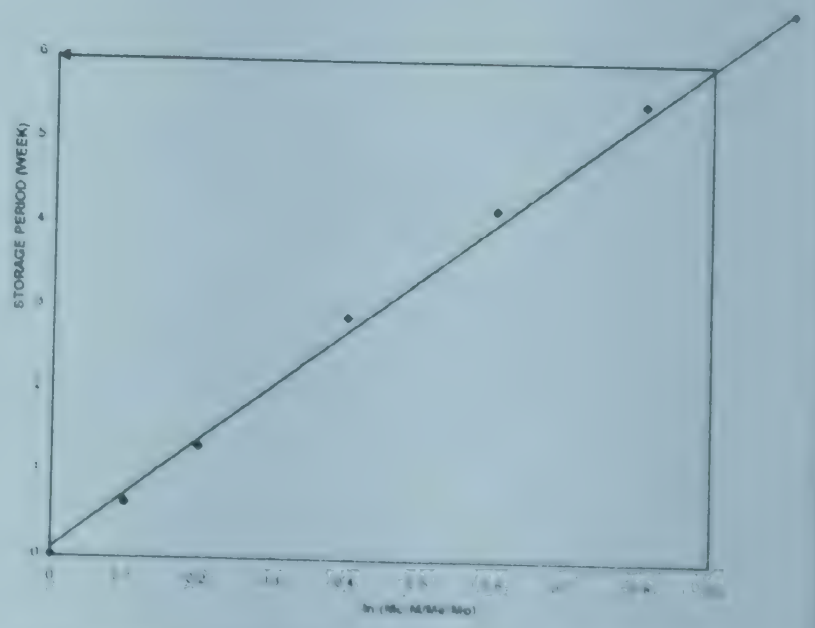

Fig. 3. Graphical representation of -ln Mc-M/Me-Mo and storage period of treated sample at 40°C 70% RH

It may be concluded that the addition of ascorbic acid in thermally processed red chilli powder helps in the retention of red colour, checks the growth of microorganism and also enriches the vitamin C content.

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RESEARCH PAPER

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Effect of Potassium Sorbate Dip-treatment in Vacuum Packaged *Lethrinus lentjan* Fillets Under Refrigerated Storage

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The effect of vaccum packaging on the shelf life of refrigerated *Lethrinus lentjan* fillets was investigated. The fillets were dip-treated with various concentrations (1.0%, 1.5% and 2.0%) of potassium sorbate, vacuum-packaged and stored in refrigeration temperature (4°C ± 1°C). The control and treated fillets were analysed periodically for biochemical (pH, TMA-N and TVB-N and FFA) and microbiological (TPC, psychrophiles, total anaerobes/facultative anaerobes, *Lactobacillus* and *Staphylococcus* counts), sensory (skin appearance, discoloration, gaping, texture and odour), quality characteristics. The shelf life periods of control packs were about 7-8 days, whereas the potassium sorbate treated fillets had an extended storage life of 20 days. Vacuum packaging alone could not prolong the shelf life of fillets in refrigeration temperature. Vacuum packaging in combination with potassium sorbate dip-treatment was found to delay the spoilage to a considerable extent by slowing down the microbial growth.

Keywords : Vacuum packaging, Shelf life, Potassium sorbate, Refrigerated storage, Quality changes.

The consumption of seafood is increasing and most consumers prefer fresh or "fresh like" seafood products. Traditionally, fresh fishery products are stored in ice or under refrigeration. Unlike frozen fish, refrigerated storage of fresh fishery products have a limited shelf life of 5-10 days (Reddy et al. 1995). Many consumers are demanding minimally processed foods that are of high order in overall quality, nutritionally superior and easy-to-prepare. For augmenting the marketing of fresh fishery products at retail level requires novel methods of packing and storage. One such method of current interest is vacuum packaging. This method of packaging can be a supplement to ice or refrigeration to delay spoilage, extend the shelf life, maintain a high quality, assure the safety and reduce economic loss of fish and fishery products.

Psychrotrophic bacteria are the major group of micro-organisms responsible for spoilage of fresh seafoods (Adams et al. 1964). Anaerobic bacterial growth is associated with spoilage of vacuum-packed seafoods (Zhuang et al. 1996). One way to retard the microbial growth is through antimicrobial agents like potassium sorbate. Debevere and Voets (1972) observed that 0.135% potassium sorbate almost completely inhibited the spoilage of cod fillets for six days by slowing the growth of bacteria capable of producing TMA. Chung and Lee (1981) reported that addition of 1% potassium sorbate extended the lag phase over six days at 0°C. Bremmer and Statham (1983) reported that potassium sorbate prolonged the shelf life of scallops to 28 days at 4°C. However, collective works on various quality aspects of vacuum-packaged fish of tropical region under refrigerated storage are scarce.

This paper reports the effects of vacuum-packaging with and without dip-treatment using potassium sorbate in various concentrations on the shelf life of refrigerated *Lethrinus lentjan* fillets as assessed by sensory, biochemical and microbiological studies.

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Materials and Methods

Fresh *Lethrinus lentjan* procured from Tuticorin fish landing centre were brought to the laboratory in 10 min. The fish samples were washed in tapwater and kept in iced condition during processing. Then, these were scaled, gutted, washed with 2 ppm chlorine water and filleted manually. Fillets (50±5g) were divided into three lots. The Lot I was air-packed and Lot II vacuum-packaged to serve as controls. The Lot III fillets were divided into three sub-lots and dip-treated in potassium sorbate solution of different concentrations viz., 1.0, 1.5 and 2.0% respectively for 30 min. (fish : treatment solution (w/v)-1:1.5) and then vacuum-packed (PSVP). Four fillets were placed in each pack. Multilayer nylon barrier film packs of 100μ thickness with oxygen transmission rate of 65 to 33.2cc/m²/day at 23°C at 80% relative humidity of size 25 × 35 cm purchased from M/s. Hitkari Industries Ltd., New Delhi were used. The LotI (control air pack-CAP) was sealed using a heat sealer. Lots II (CVP) and III (PSVP) were vacuum-packed at 1 bar pressure using a vacuum sealing machine, (Make Alfa laval, Kramergrebe; Model quick 2000A Germany). The packs were stored in a refrigerator (4±1°C). Samples were drawn from each lot at regular intervals of 3 days for analysis.

Biochemical analysis : The pH was measured after homogenising 10 g samples in 50 ml of distilled water using Systronics digital pH meter 335. TMA-N and TVB-N contents were estimated by Conway microdiffusion method (Beaty and Gibbons 1937). Free fatty acids were estimated by titrimetric method (Ke et al. 1976).

Microbiological analysis : Total plate count, psychrophiles, total anaerobes/facultative anaerobes, *Lactobacillus* and *Staphylococcus* counts were determined as per APHA (1976). Soybean casein digest agar was used to enumerate total plate count (37°C and 48 h) and psychrophiles. (5°C, 5 days) Population of anaerobes/facultative anaerobes was determined by 3 tube MPN technique using fluid thioglycollate medium. The tubes were incubated at 37°C for 48 h. *Lactobacillus* MRS

TABLE 1. SENSORY SCORE SHEET FOR REFRIGERATED *LETHRINUS LENTJAN* FILLETS

Grade points	5	4	3	2	1
Sensory attributes					
Skin Appearance	Characteristic Bright	Bright	Slight loss of bright colour	Moderately dull	Faded, dull, slimy
Texture	Firm	Slightly soft	Moderately soft	Soft	Very soft
Gaping	Absent	Very slight	Slight	Moderate	Severe
Odour	Characteristic, seaweedy, sealike	Loss of characteristic odour, condensed milk	Curdy, fruity (tart)	Curdy, sour, acidic	Putrid, sulphide
Discoloration	Absent	Very slight red tinge	Red coloration in a portion of fillet	Spreading of red coloration in major portion of fillet	Complete reddening of fillet

agar medium was used to enumerate *Lactobacillus* sp. Baird Parker agar was used to enumerate *Staphylococci* count (37°C for 48 h.).

Sensory evaluation : Various sensory characteristics such as skin appearance, discoloration, gaping, texture and odour were evaluated by 5 panelists. Five point scale suggested by Brenner (1985) was modified (Table 1) and used. Scores for each attribute were pooled and the mean panel scores are presented.

Statistical analysis : Analysis of variance (ANOVA) technique was followed (Snedecor and Cochran 1962) to find out the significant difference among samples in relation to biochemical, microbiological and sensory characteristics.

Results and Discussion

The initial pH of the fillet was 6.33 and on storage, the pH values increased significantly ($P<0.01$) in both control (CAP and CVP) and treatment packs (PSVP) (Table 2). The increase in pH of the control packs was faster than in treatment packs. The increse in pH may be attributed to the production of volatile base compounds by bacterial activity (Cann et al. 1983). Reddy et al (1995) reported that increase in surface pH of 100% air packaged tilapia fillets stored at 4°C, 8°C and 16°C may be partly attributed to the production of volatile basic compounds such as ammonia by fish spoilage bacteria. During the end of storage period, there was a decline in pH of the treatment packs. The reduction in pH at the end of storage might be due to the growth of Lactic Acid Bacteria to a level of 10^6/g. Meekin et al (1982) also reported decline in pH of vacuum-packed sand flat head fillets stored at 4°C after 6 days of storage.

Trimethyl amine is a product of bacterial spoilage and is often used as an index to assess the keeping quality of seafood products (Hebard et al. 1982). The TMA-N contents of fish fillets showed an increasing trend in control packs ($P<0.05$) and in treatment ($P<0.01$) packs (Table 2). TMA-N was found to be absent initially and its presence was observed on the third day sampling in control packs and on the 6th day in PSVP 1.0%, PSVP 1.5% and on the 9th day on PSVP 2.0% (Table 2). The delay in the formation of TMA-N can be attributed to lower bacterial load in the samples. Inhibitory effect of potassium sorbate over the growth of bacteria may be the reason for the delayed formation of TMA-N. Connel (1975) reported that 10-15 mg% of TMA-N was usually regarded as the limit of acceptability for human consumption in iced fish. Curran et al (1980) has demonstrated that much larger amounts of TMA-N are formed in tropical fishes. Results of the present study are in agreement with the above report. The TMA-N contents of samples at the time of spoilage were in the range of 20-40 mg%. Parkin et al (1981) stated that TMA provided an adequate quality index for air-packaged as well as modified atmospheric packed rockfish fillets stored at 35°F and could be used as a quality index for refrigerated fish fillets.

Total volatile base comprises mainly, trimethyl amine (TMA) and ammonia (NH_3) that are produced by both bacterial and endogenous enzymes (Lannelongue 1980). The TVBN values were found to increase in all samples with storage period ($P<0.05$) in control packs (CAP and CVP) and ($P<0.01$) in treatment packs (PSVP) (Table 2). The TVB-N contents increased from initial 13.72 to 19.00 mg% in CAP and 77.84 mg% in CVP on the day of rejection. These values were much higher than those of treatment packs on the same day. Low levels of TVB-N in treatment samples were due to either a reduced bacterial population or decreased capacity of bacteria for oxidative deamination of non-protein nitrogen compounds or both (Banks et al. 1980). Potassium sorbate has also been reported to inhibit TVB-N formation in cod fillets by Debevere and Voets (1972).

The oxidation and hydrolysis of lipid in fishes during storage cause quality deterioration. Lipid autolysis, an enzymatic hydrolysis results in the formation of free fatty acids (Huss 1988). The free fatty acid (FFA) values increased significantly ($P<0.01$) with storage period in potassium sorbate treated samples but not controls (Table 2). However, the amounts of free fatty acids in the treated samples were lower than those of control on the day of rejection of control samples (CAP and CVP). The increases in FFA contents in CAP were found to be more than those of CVP and PSVP samples. This suggested that vacuum packaging would prevent the rapid formation of FFA. However, due to the prolonged storage of treated samples, there were increases in FFA contents. Huang et al (1991) observed that vacuum packaging did slow down the lipid hydrolysis in farm-raised channel catfish in iced storage. Huang et al (1992, 1994) further reported that vacuum skin packaged channel catfish fillets had significantly lower FFA than overwrapped fillets stored at 4°C, suggesting that FFA may not be considered as a reliable index of spoilage in vacuum-packed refrigerated fish fillets.

TABLE 2. BIOCHEMICAL CHANGES OF FISH FILLETS DURING REFRIGERATED STORAGE

Parameters	Storage period, days	CAP	CVP	PSVP 1.0%	PSVP 1.5%	PSVP 2.0%
pH	0	6.33 ± 0.06	6.33 ± 0.06	6.33 ± 0.06	6.33 ± 0.06	6.33 ± 0.06
	3	6.38 ± 0.01	6.39 ± 0.08	6.35 ± 0.07	6.34 ± 0.20	6.34 ± 0.11
	6	6.69 ± 0.04	6.65 ± 0.03	6.50 ± 0.07	6.51 ± 0.09	6.45 ± 0.14
	9	6.88 ± 0.10	6.80 ± 0.07	6.55 ± 0.05	6.53 ± 0.11	6.50 ± 0.09
	12	ND	ND	6.72 ± 0.10	6.70 ± 0.07	6.58 ± 0.07
	15	ND	ND	6.80 ± 0.11	6.78 ± 0.08	6.70 ± 0.70
	18	ND	ND	6.72 ± 0.10	6.73 ± 0.02	6.68 ± 0.08
	21	ND	ND	ND	ND	6.67 ± 0.04
TMA-N,	0	0.00	0.00	0.00	0.00	0.00
mg %	3	9.50 ± 0.01	7.28 ± 0.07	0.00	0.00	0.00
	6	25.20 ± 0.01	19.60 ± 0.11	11.76 ± 0.18	7.28 ± 0.04	0.00
	9	45.92 ± 0.15	35.28 ± 0.25	17.36 ± 0.09	8.40 ± 0.03	5.60 ± 0.14
	12	ND	ND	18.20 ± 0.16	11.60 ± 0.03	8.40 ± 0.15
	15	ND	ND	23.52 ± 0.21	19.60 ± 0.11	12.06 ± 0.09
	18	ND	ND	30.62 ± 0.18	22.40 ± 0.10	15.46 ± 0.08
	21	ND	ND	ND	ND	19.60 ± 0.09
TVB-N,	0	13.72 ± 0.14	13.72 ± 0.14	13.72 ± 0.14	13.72 ± 0.14	13.72 ± 0.14
mg %	3	22.40 ± 0.12	30.80 ± 0.09	17.50 ± 0.05	16.80 ± 0.08	13.00 ± 0.03
	6	50 42 ± 0.09	68.88 ± 0.12	25.20 ± 0.08	22.40 ± 0.09	14.24 ± 0.04
	9	91.00 ± 0.10	77.84 ± 0.24	26.88 ± 0.02	25.20 ± 0.11	17.26 ± 0.06
	12	ND	ND	27.16 ± 0.10	26.88 ± 0.08	22.40 ± 0.11
	15	ND	ND	35.84 ± 0.12	27.44 ± 0.06	24.28 ± 0.12
	18	ND	ND	42.00 ± 0.11	38.92 ± 0.05	30.46 ± 0.10
	21	ND	ND	ND	ND	34.90 ± 0.06
FFA,	0	0.150 ± 0.02	0.150 ± 0.02	0.150 ± 0.02	0.150 ± 0.02	0.150 ± 0.02
% of oleic acid	3	0.885 ± 0.02	0.543 ± 0.03	0.172 ± 0.01	0.176 ± 0.01	0.133 ± 0.02
	6	1.230 ± 0.02	0.791 ± 0.00	0.198 ± 0.02	0.179 ± 0.01	0.214 ± 0.01
	9	3.800 ± 0.10	1.003 ± 0.01	0.645 ± 0.04	0.394 ± 0.04	0.516 ± 0.03
	12	ND	ND	0.816 ± 0.03	0.743 ± 0.07	0.986 ± 0.04
	15	ND	ND	1.983 ± 0.01	1.543 ± 0.04	1.410 ± 0.06
	18	ND	ND	2.420 ± 0.04	2.540 ± 0.03	1.143 ± 0.03
	21	ND	ND	ND	ND	2.340 ± 0.01

Note : Values indicate mean of three estimations
ND : Not Done

Microbial growth in fresh seafood is the main factor associated with quality deterioration, spoilage and economic loss (Zhuang et al. 1996). The total plate count was found to increase significantly ($P<0.01$) with storage period (Fig. 1) The lower count in PSVP fillets on the day of sensory rejection of control packs might be due to the preservative action of potassium sorbate. Bremmer and Statham (1983) reported suppression of spoilage by addition of potassium sorbate to vacuum-packed scallops under refrigeration storage at 4°C. The generation time of mesophiles in CAP was 16.13 h as against 18.70 h for CVP, which indicated that the vacuum condition developed some stress over mesophiles, thereby reduction in proliferation rate.

Psychrotrophic bacteria are the major group of microorganisms responsible for spoilage of fresh seafoods (Adams et al. 1964). The psychrophilic count increased significantly ($P<0.01$) with storage period in all the samples (Fig. 2). The counts in the PSVP samples were found to be significantly lower compared to CAP and CVP. This again might be due to the inhibition of psychrophiles by potassium sorbate. Generally, the counts were in the range of 10^7 to 10^8 in all the samples on the day of sensory rejection. Dalgaard et al (1993) reported that microorganisms with psychrophilic characters were dominating the microflora of spoiled fillets. Vacuum packaging prolongs the shelf life of fresh fish by suppressing the growth of psychrotrophic aerobic organisms associated with spoilage (Clingman and Hooper 1986).

In all the samples, there were increases ($P<0.01$) in LAB counts during storage (Fig. 3). In the treatment packs, the counts were at a level of 10^6 on the 12th day, after which the counts remained constant until the day of rejection. This may be attributed to the supression of its growth by other microorganisms. Meekin et al (1982) have reported that unless LAB are deliberately added, it is unlikely that they will proliferate on vacuum-packed fillets sufficiently to compete effectively with gram-negative facultative anaerobes. Further, its association

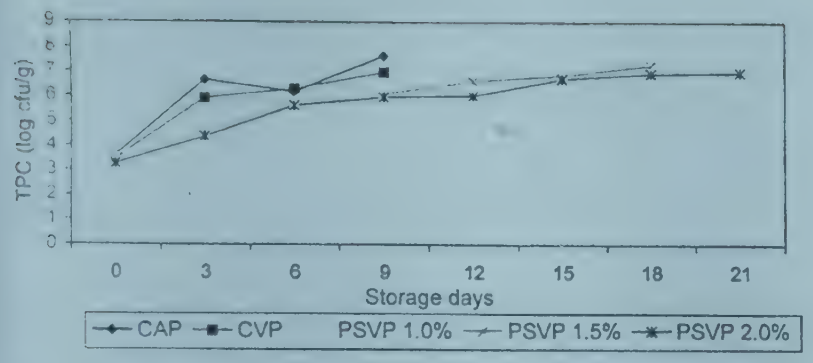

Fig. 1. Changes in TPC (cfu/g) of fish fillets during refrigerated storage

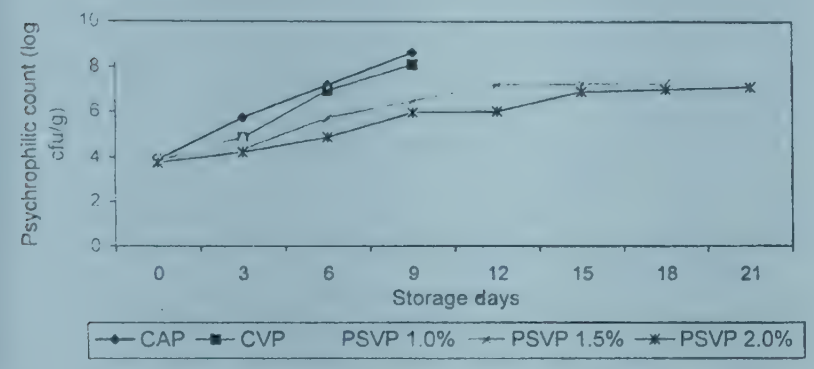

Fig. 2. Changes in psychrophilic count (cfu/g) of fish fillets during refrigerated storage

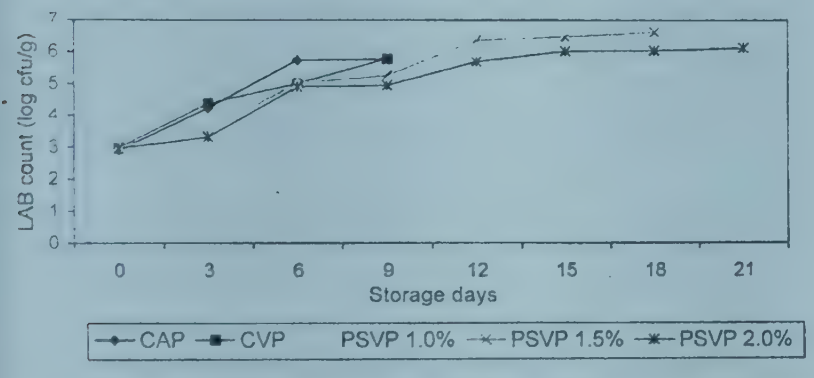

Fig. 3. Changes in LAB count (cfu/g) of fish fillets during refrigerated storage

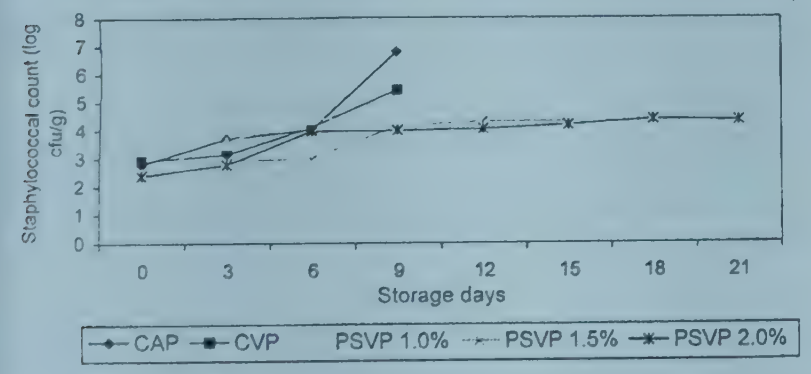

Fig. 4. Changes in staphylococcal count (cfu/g) of fish fillets during refrigerated storage

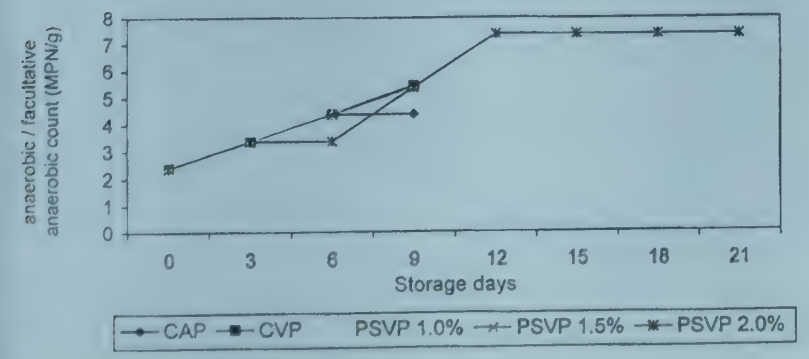

Fig. 5. Changes in anaerobic/facultative anaerobic bacterial count (MPN/g) of fish fillets during refrigerated storage

with spoilage in vacuum-packed fishes did not lower the pH in fish fillets. However, its proliferation had a significant impact over the odour, resulting in the development of milky, curdy and fermented smell. The *staphylococcal* counts increased significantly in control and treatment ($P<0.01$) packs (Fig. 4). The counts in treated packs were comparatively lower by two log units than the control packs (CAP and CVP). The growth of *S. aureus* in refrigerated temperature is not unusual and has been reported by Palumbo (1986) and Shanmugam et al (1993). The lower *staphylococcal* counts might be attributed to the inhibitory effect of potassium sorbate. This agreed with the result of Tompkin et al (1974), who reported that sorbate inhibited the growth of *Staphylococcus aureus* in cooked, uncured sausage.

Anaerobic bacterial growth is associated with spoilage of vacuum-packaged seafoods (Zhuang et al. 1996). There was a significant increase in anaerobes/facultative anaerobes in control packs ($P<0.05$) and in treatment packs ($P<0.01$) (Fig. 5). The counts in CAP were found to be lower than those of vacuum packs, indicating that vacuum package created, conditions suitable for the growth of anaerobes/facultive anaerobes. From the 12th day onwards, the counts remained almost constant. Reddy et al (1997) reported that in modified atmospheric packaged catfish fillets stored at 4°C, anaerobic microbes reached the high during the first 13-15 days of storage and thereafter, remained constant until spoilage. This might be due to the domination by other bacterial flora. This could be compared to psychrophilic counts where the psychrophiles proliferated rapidly during the later stages of storage.

In this experiment, the fillets are considered to be acceptable for human consumption, until the sensory score reached 3 with the following characteristics (Table 1), slight loss in bright colour of skin, moderately soft texture, slight gaping with curdy/fruity odour and discoloration in a portion of fillet. The shelf life of CAP was found to be about 7 days. Meekin et al (1982) reported that packed and refrigerated (4°C) sand flat head spoiled in 8-9 days. The shelf life of control vacuum pack was found to be 8 days. Several authors have reported various shelf life periods for different fishes: excellent quality vacuum packed cod fillets (7 days), (Huss 1971) and vacuum packed sand flat head fillets (8-9 days) (Meekin et al. 1982) at 4°C. The shelf life periods of potassium sorbate treated packs were found to be significantly higher than those of controls with shelf life increasing with increase in potassium sorbate concentration. The shelf life of control pack was found to be lesser than that of vacuum packed potassium sorbate treated scallops, whose shelf life was 28 days (Bremmer and Statham 1983) at refrigerated storage (4°C).

The results of the present study revealed that vacuum packaging of fish fillets alone, without preservative treatment, would not be of much use, under said experimental conditions. Several authors have demonstrated successfully the multiple barrier technology or hurdles concept i.e., combination or superimposing several factors at sub-inhibitory concentrations that can effectively control microorganisms in refrigerated seafood (Leistner and Gorris 1995; Scott 1989). In this regard, results of this study clearly suggested that the combination of different

factors viz., vacuum packaging, treatment with preservative 9potassium sorbate) and storage at refrigerated temperature could be used to prolong the shelf life of the vacuum-packed fish fillets to a great extent.

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RESEARCH PAPER

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Studies on Occurrence and Distribution of *Acinetobacter* spp. and Other Gram-negative Bacteria from Meat

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A total of 98 bacterial strains were recovered employing 5 different media from 12 retail meat samples, belonging to 3 genera consisting of 28 *Acinetobacter* (28.57%), 23 *Moraxella* (23.4%) and 47 *Pseudomonas* (47.96%) in different conditions. *Acinetobacter* was found to be the dominant organism (38.25%), followed by *Pseudomonas* (32.72%) in meat stored at refrigerator temperature. Though *Pseudomonas* was dominant in fresh (43.6%) and spoiled meat (42.9%) and meat stored at freezer temperature (-20°C for 20 days) (83.9%), while *Acinetobacter* was found to be the next dominant bacterium 31.6%, 18.5% and 10.39%, respectively from the above three categories of meat. Holton's medium without cefsulodin was found to be the suitable medium for the recovery of *Acinetobacter* from meat.

Keywords : *Acinetobacter, Moraxella, Pseudomonas,* Meat, Distribution, DNA transformation, Holton's medium, Refrigerator temperature.

Acinetobacters have been reported by numerous investigators to be a part of the normal flora of fresh meats (Dainty et al. 1983; Eribo and Jay 1985; Davis and Board 1998). *Acinetobacter/Moraxella* group constitutes an important group of microorganisms, though *Pseudomonas* is the pre-dominant genus of bacteria in fresh meats, allowed to spoil aerobically at refrigerator temperatures (Ayres 1960a, b; Gill and Newton 1980; Eribo et al. 1985; Narasimha Rao and Sreenivasamurthy 1985; Gennari et al. 1989). Because of the morphological, biochemical and colonial similarities of these genera, many workers could not separate them but designated them as *Acinetobacter-Moraxella,* even though the former being oxidase negative and the latter, oxidase positive (Holton 1983; Eribo and Jay 1985; Eribo et al. 1985; Gennari et al. 1989, 1992).

There are few reports on microbial spoilage of meat at higher temperatures (Gill and Newton 1980). The practice of slaughter, holding and meat sale under commercial conditions in a tropical country like India is different from that in Western countries. In many parts of India, the goat and sheep carcasses are brought to the retail shops from the local slaughter-house within 30 min after slaughter. The meat remains for 18 to 20 h in the retail shops. In such a situation, it is observed that some of the carcasses would remain in the retail shops until everything is sold. Hence, there is a need to know the bacteria associated and the shelf life of meat under such conditions.

Acinetobacter plays an important role in the spoilage of meat and foods under various conditions (Jay and Shelf 1978; Banwart 1981; Choudhari et al. 1990; Jay 1991; Frazier and Westhoff 1995). Various media previously suggested for the recovery of *Acinetobacter* spp. have been found to be unsatisfactory for various reasons (Mandel et al. 1964). In recent years, *Acinetobacter* has emerged as an important nosocomial pathogen and it is one of the most resistant bacteria to antibiotics and metals (Deshpande et al. 1993; Chopade et al. 1994; Dhakephalkar and Chopade 1994; Deshpande and Chopade 1994). Food is the important source of transmission of pathogens to man and hence the work on the presence of *Acinetobacter* in food warrants further investigations. In view of this background, the present study was undertaken to investigate the occurrence and distribution of *Acinetobacter* spp. and related Gram-negative bacteria in both fresh and aerobically spoiled meats at room temperature as well as stored at refrigerator (4°C) and freezer (-20°C) temperatures and to find out the suitability of medium for the recovery of *Acinetobacter* from meat.

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Materials and Methods

Collection and processing of meat samples : Twelve fresh meat samples were collected from different retail meat shops in Pune city, India during July 1998-September 1998. During this period, the average temperature range used to be between 26 and 31.8°C and the relative humidity between 61 and 76%. For each sample, 1 kg fresh meat was collected in sterile polythene bags and carried to the laboratory within half an hour after slaughtering. The fresh meat samples were: of bright-red colour with fleshy-smell, temperature ranging from 30 to 32°C. In the laboratory, all the fresh meat samples were divided into four categories (each 250 g in sterile polythene bags) aseptically for the aerobic bacterial study of: (a) Fresh meat; (b) Meat kept at room temperature, spoilage (0 to 24 h) (c) Meat kept at refrigerator (4°C for 10 days) and (d) Meat kept at freezer temperature (-20°C for 20 days).

For open air spoilage, meat sample-(b) kept at room temperature (26 to 32°C) covered by 0.2 mm pore size mesh and was allowed to proper aeration but strictly inhibited from entrance of any fly. To investigate the shelf life, meat samples were processed for aerobic culture through the time range of 0 h, 6 h, 10 h, 14 h, 18 h, 20 h and 24 h.

Culture media for isolation of Acinetobacter *and other Gram-negative bacteria :* Five culture media were employed for the recovery of *Acinetobacter* spp. and other related genera. Fifty grams from each meat sample were taken in 250 ml conical flasks and suspended in 50 ml 0.85% sterile saline (pH 7) and shaken for 20 min at 250 rpm at room temperature. The contents of each flask were serially diluted in sterile 0.85%

saline (pH7) up to eight-fold dilutions and 100 µl of each dilution was plated on duplicate plates of Holton's selective medium (Holton 1983) without cefsulodin antibiotic; Cysteine Lactose Electrolyte Deficient agar (CLED) (HiMedia, Mumbai, India) Mandel's medium (Mandel et al. 1964), *Acinetobacter* Minimal Medium (AMM) agar (Chopade et al. 1985), and Standard Plate Count Agar (SPCA). The plates were incubated aerobically at 28°C for 24 to 96 h. All the bacterial colonies were selected randomly from all media plates and counted as the square root of the mean count of duplicate plates. Every colony type obtained was purified and transferred on to CLED agar slants separately and the cultures were given a monthly transfer on the same culture medium slants. All the cultures were preserved on CLED agar slants at 4°C.

Enrichment of Acinetobacter spp. *:* Enrichment of *Acinetobacter* was carried out employing Baumann's enrichment medium (Choudhari et al. 1990). In brief: 50 g from each meat sample was taken in a 250 ml conical flask and suspended in 50 ml 0.85% sterile saline (pH 7) and shaken for 20 min at 250 rpm at room temperature. One ml of meat suspension (meat-saline extract) was inoculated into the 50 ml Baumann's medium for enrichment (Baumann 1968). It was also inoculated into 50 ml *Acinetobacter* Minimal Medium (AMM) (Chopade et al. 1985). The flasks were incubated at room temperature (28 to 30°C) at 250 rpm for 24 to 48 h and the turbidity was observed in each flask. The growth from both flasks was serially diluted (up to 10^{-8}) and 100 µl of each dilution was plated on all 5 different media and incubated at 28 to 30°C up to 96 h and growth of *Acinetobacter* was observed.

Characterization of Acinetobacter *and other Gram negative bacteria :* All the isolates were tested for the following characters : colony and cell morphology, motility, Gram staining, capsule staining by Indian ink (Gerhardt et al. 1994), oxidase, catalase, fermentation of glucose, fermentation of lactose, acid from D-glucose, hydrolysis of gelatine and Tween 80, haemolysis, nitrate-reduction and H_2S production. Lipase, protease, amylase, urease and DNAse activity were carried out by standard methods (Gerhardt et al. 1994; Collins et al. 1995).

Chromosomal DNA transformation assay : The method of chromosomal DNA transformation assay as reported by Juni (1972) and Deshpande and Chopade (1994) was followed with some modifications. Chromosomal DNA was isolated and purified by the method described by Marmur (1961) from *Acinetobacter* meat isolates and then used as a source of transforming DNA. The recipient strain used for transforming DNA was the naturally competent strain of *Acinetobacter calcoaceticus* BD413 trpE27, which was grown on Brain-Heart Infusion agar (BHI) (Hi Media) before transformation for 2 to 3 times at 28°C. *Acinetobacter* minimal medium (AMM) with 2% Noble agar (Difco, USA) with 50 µg/ml tryptophan was employed for the growth of transformants. Standard strain of *Acinetobacter calcoaceticus* MTCC 1271; *A. lwoffii* MTCC 496; *A. calcoaceticus* MTCC127 and *Escherichia coli* K12 HB 101, and *E. coli* DH5α were used as positive and negative control. The AMM agar plates were incubated at 28°C for 24-120 h. The transformants were purified on the same medium and growth of transformants was confirmed.

Statistical analysis : All the bacterial colonies were selected randomly from all media plates and counted as the square root of the mean count of duplicates. The mean (×) values of total count and their standard deviations (SD) were subjected for their proper distribution (Fletcher et al. 1982).

Results and Discussion

On Holton's medium, *Acinetobacter* and *Moraxella* colonies developed pink colour after overnight incubation at 28°C. However, after 48 to 96 h incubation at 28°C, colonies became pinkish to cream and after overnight incubation, the sizes of the colonies were 1-2 mm but with further incubation, colony size also increased up to 4-8 mm. Colonies were high convex to low convex in elevation, entire, smooth, moist and highly mucoid in consistency. As compared to *Moraxella*, *Acinetobacter* colonies were much more mucoid, whereas *Pseudomonas* colonies were normally non-mucoid, small in size (0.5-2 mm) and of pale pink colour. On Mandel's medium, *Acinetobacter* colonies were faint green to creamy, whereas *Moraxella* colonies were blue to faint green. On CLED agar plates, both *Acinetobacter* and *Moraxella* were faint green to cream and showed highly mucoid, translucent, round, smooth, opaque and 1-4 mm in diameter, whereas *Pseudomonas* colonies were small (0.5-2 mm), circular spreading type greenish colony. On AMM agar plates, *Acinetobacter* colonies were small (0.5-2 mm), white to cream, after further incubation, colony sizes also increased up to 4 mm. *Acinetobacter* colonies were circular, smooth, mucoid, entire, translucent and cream colour on SPCA plates, whereas *Moraxella* colonies were round-spreading, entire and yellowish-cream and *Pseudomonas* colonies were bluish green.

Both *Acinetobacter* and *Moraxella* were non-motile, some isolates showed the twitching motility, Gram-negative cocco-bacilli, single, pairs and in chains of 1 to 5 cells, capsulated and all were catalase positive. Members of genus *Acinetobacters* strains were oxidase negative, whereas, Morexellae were oxidosepositive. The *Pseudomonas* strains were motile, rods, Gram-negative, non-capsulated, non-spore former, non-fermenter, oxidase and catalase positive. There was little difference in colony morphology between the growth of *Acinetobacter* and *Moraxella* on all five media. Also, there was no significant difference in cell morphology and their twitching motility. However, members of genus *Pseudomonas* exhibited significant difference. Similar observation has been reported earlier (Thornley 1967; Shewan and Murray 1979; Shaw and Latty 1988).

Among the 28 *Acinetobacter* strains, 15 produced acid from D-glucose, in O/F test, 18 utilised glucose oxidatively, 2 fermentatively and 8 facultatively, whereas, 10 strains were found lactose fermenter. In nitrate reduction test, 3 *Acinetobacter* strains showed positive reaction and remaining were negative. Eight strains grew on 6% NaCl concentration. Sixteen were Tween 80 hydrolysers, 3 were gelatinase producer and the same 3 strains showed β-haemolysis activity. Among the 5 enzymatic tests, 15 strains of *Acinetobacter* were found to be lipase positive, 8 were protease, 2 were amylase, and 4 strains were urease positive. Interestingly, 4 strains of *Acinetobacter* exhibited DNAse activity. In this study, none of the strains was

found H_2S producer. However, many of them showed the strong lipase and protease activities. These findings agreed with that reported by earlier workers (Shewan and Murray 1979; Gibbs et al. 1982; Gram et al. 1987; Gennari et al. 1989). *Moraxella* also showed the similar characteristics in biochemicals as those found in *Acinetobacter.*

Out of 98 bacterial strains obtained from meat, 28 (28.57%) showed characters similar to those of the members of genus *Acinetobacter,* 23 (23.47%) *Moraxella* and 47 (47.96%) *Pseudomonas.* Out of 40 isolates, 28 strains of *Acinetobacter* were confirmed by the chromosomal DNA transformation assay (Juni 1972). Most of the transformants grew well within the first 48 h of incubation at 28°C. No growth was observed for the rest of the isolates and *E. coli* strains were used as negative control and did not transform even after 120 h of incubation at 28°C.

Distribution of bacterial isolates from fresh meat in all five culture media, the highest counts of *Acinetobacter* spp. were found on standard plate count agar (9.5 × 10^8/g), followed by Holton's medium (6.5 ×10^8/g), in which the highest number of *Moraxella* and *Pseudomonas* were found on SPCA (8.1 × and 13.5 × 10^8/g) and followed by those on CLED (6.3 × 10^8/g) and Holton's (11.6 × 10^8/g), respectively (Table 1). In open air, spoilage of meat at room temperature, both *Acinetobacter* and *Pseudomonas* showed the highest total counts on SPCA (2.5 × and 8.6 × 10^8/g) and the next highest count was found on Holton's (2.4 and 7.0 × 10^8/g, respectively), in which *Moraxella* showed the highest count on Holton's (6.54´10^8/g). In meat stored at refrigerator, the bacterial counts (*Acinetobacter, Moraxella* and *Pseudomonas*) were found to be the highest on SPCA (10.7, 9.2 and 12.8 × 10^8/g respectively) and the next highest counts were found on Holton's medium (9.6, 8.5 and 11.6 × 10^8/g, respectively). In meat stored at freezer temperature, the highest number of *Acinetobacter* was recovered from Holton's medium (1.3 × 10^8/g) in which *Moraxella* and *Pseudomonas* were recovered from SPCA (1.1 and 12.4 × 10^8/g) and in both these cases, the next important medium was found to be Holton's (0.76 and 11.23 × 10^8/g) (Table 1). These results showed that *Pseudomonas* was the most dominant organism and the next dominant one was *Acinetobacter* isolated from the different conditions in meats. These findings also were in confirmity with those of Lahellec et al (1975); Gill and Newton (1977, 1980); Eribo and Jay (1985); Shaw and Latty (1988).

Holton's medium without cefsulodin was found to be the most satisfactory selective medium for the recovery of *Acinetobacter* as compared to other culture media. Holton (1983) has also reported similar finding. CLED agar was also found to be a good medium as compared to other medium. The lactose fermenter group of *Acinetobacter* and *Moraxella* was easily detected by us yellowish colony on CLED agar. Among the four categories of meat, the total highest bacterial counts were found in open air spoiled meat at room temperature, where the dominant organism was *Pseudomonas* (25.1 × 10^8/g followed by *Moraxella* (22.6 × 10^8/g) and *Acine-tobacter* (10.8 × 10^8/g)

TABLE 1. DISTRIBUTION OF *ACINETOBACTER* SPP. AND OTHER GRAM NEGATIVE BACTERIA FROM MEAT UNDER VARIOUS CONDITIONS

Bacteria	Number of Isolates (cfu/g) in Different Culture Media **															
	Holton's*			Mandel's			CLED			AMM			SPCA			Total
	TVC	$\overline{X}$	SD	TVC	$\overline{X}$	SD	TVC	$\overline{X}$	SD	TVC	$\overline{X}$	SD	TVC	$\overline{X}$	SD	
Fresh meat (cfu × 10^8)β12																
Acinetobacter spp.	6.5	0.54	0.95	5.8	0.48	0.55	6.4	0.53	0.59	3.4	0.34	0.44	9.5	0.79	0.95	31.65
Moraxella spp.	4.8	0.40	0.63	5.2	0.43	0.58	6.3	0.52	0.51	ND	ND	ND	8.1	0.67	0.68	24.55
Pseudomonas spp.	11.6	0.96	2.2	8.5	0.71	1.00	9.9	0.82	1.60	NG	NG	NG	13.5	1.10	1.40	43.69
Spoiled meat (cfu × 10^8)β8																
Acinetobacter spp.	2.4	0.30	0.22	1.6	0.20	0.16	2.1	0.26	0.29	2.1	0.29	0.51	2.5	0.32	0.28	11.86
Moraxella spp.	6.5	0.81	0.76	5.6	0.70	1.00	5.2	0.65	0.66	ND	ND	ND	5.2	0.65	0.89	22.60
Pseudomonas spp.	7.0	0.87	0.77	3.2	0.40	0.76	6.2	0.78	0.67	NG	NG	NG	8.6	1.10	0.99	25.14
Meat stored at 4°C for 10 days (cfu × 10^8)β11																
Acinetobacter spp.	9.6	0.87	0.82	6.3	0.57	0.51	8.5	0.77	0.72	8.3	0.75	0.69	10.7	0.97	0.95	43.58
Moraxella spp.	8.5	0.77	0.60	7.6	0.69	0.37	7.6	0.69	0.41	ND	ND	ND	9.2	0.84	0.43	33.00
Pseudomonas spp.	11.6	1.00	1.00	4.5	0.40	0.65	8.3	0.75	0.76	NG	NG	NG	12.8	1.16	1.13	37.23
Meat stored at -20°C for 10 days (cfu × 10^8)β10																
Acinetobacter spp.	1.36	0.13	0.19	0.78	0.07	0.12	0.82	0.08	0.09	1.1	0.11	0.11	1.0	0.10	0.09	5.15
Moraxella spp.	0.76	0.07	0.13	0.49	0.04	0.06	0.50	0.05	0.06	ND	ND	ND	1.0	0.10	0.12	2.78
Pseudomonas spp.	11.20	1.10	1.20	7.70	0.77	0.76	10.30	1.00	0.61	NG	NG	NG	12.4	1.20	0.66	41.63

** = This values are average of three experiments; *= without cefsulodin; CLED=Cysteine Lactose Electrolyte Deficient; AMM=*Acinetobacter* Minimal Medium; SPCA=Standard Plate Count Agar; β=No of Sample; TVC= Total Viable Count; $\overline{X}$= Mean; SD=Standard Deviation; ND=Not Done; NG=No Growth

TABLE 2. FREQUENCY OF *ACINETOBACTER* SPP. AND OTHER GRAM NEGATIVE BACTERIA FROM MEAT IN DIFFERENT CULTURE MEDIA (cfu × 10^8/g)**

Bacteria	Total Isolates	Holton's* TVC	Holton's* %	Mandel's TVC	Mandel's %	CLED TVC	CLED %	AMM TVC	AMM %	SPCA TVC	SPCA %
Fresh meat											
Acinetobacter spp.	31.65	6.53	20.6	5.80	18.3	6.41	20.2	3.4	10.7	9.5	30.0
Moraxella spp.	24.55	4.85	18.7	5.25	21.4	6.35	25.8	ND	ND	8.1	32.9
Pseudomonas spp.	43.69	11.60	26.6	8.58	19.6	9.95	21.9	NG	NG	13.6	31.0
Spoiled meat (at room temperature)											
Acinetobacter spp.	1085	243	22.4	162	14.9	210	19.4	214	19.7	256	23.6
Moraxella spp.	2260	654	28.9	563	24.9	520	23.0	ND	ND	523	23.2
Pseudomonas spp.	2514	700	27.8	326	12.9	625	24.8	NG	NG	863	32.3
Meat stored (at 4°C for 10 days)											
Acinetobacter spp.	43.50	9.60	22.1	6.35	14.6	8.56	19.7	8.3	19.1	10.80	24.7
Moraxella spp.	33.00	8.55	25.9	7.67	23.2	7.60	23.0	ND	ND	9.25	28.0
Pseudomonas spp.	37.27	11.60	31.2	4.50	12.1	8.30	22.3	NG	NG	12.80	34.4
Freezer meat (-20°C for 10 days)											
Acinetobacter spp.	51.50	1.36	26.4	0.78	15.2	0.82	15.9	1.1	21.7	1.07	20.8
Moraxella spp.	2.78	0.76	27.5	0.50	17.8	0.51	18.2	ND	ND	1.02	36.5
Pseudomonas spp.	41.63	11.2	26.9	7.70	18.5	10.30	24.7	NG	NG	12.40	29.8

** = These values are average of three experiments; *= without cefsulodin; CLED=Cysteine Lactose Electrolyte Deficient; AMM=*Acinetobacter* Minimal Medium; SPCA=Standard Plate Count Agar; TVC= Total Viable Count; SD=Standard Deviation; ND=Not Done; NG=No Growth

(Table 1), while meat stored at freezer temperature showed the lowest bacterial counts as compared to fresh, spoiled and refrigerators meat. The total *Acinetobacter* counts dominated over *Pseudomonas* and *Moraxella* on refrigerator meat.

The frequencies of *Acinetobacter, Moraxella* and *Pseudomonas* in different culture media are indicated in Table 2. In fresh meat, isolates of *Acinetobacter, Moraxella* and *Pseudomonas* showed the highest percentage on SPCA (30 %, 32.9% and 31%, respectively) and the next highest percentage was found on Holton's medium (20.6% and 26.6%) except *Moraxella* (25.8%) on CLED. In room temperature, spoiled meats *Acinetobacter* and *Pseudomonas* also showed the highest percentage on SPCA (23.6% and 32.3% respectively) and second highest percentage was on Holton's medium (22.4% and 27.8%, respectively), while *Moraxella* was found to be the highest percentage on Holton's (28.9%). The isolates of *Acinetobacter, Moraxella* and *Pseudomonas* from refrigerator meats showed the highest percentages on SPCA (24.7%, 28% and 34.4%, respectively) and the next highest percentages were found on Holton's medium (22.1%, 25.9% and 31.2%, respectively). The percentage recovery of *Acinetobacter* from freezer meats was found to be the highest on Holton's medium (26.4%), while *Moraxella* and *Pseudomonas* were found to be in highest percentages on SPCA (36.5% and 29.8% respectively) (Table 2).

The percentage of bacterial isolates in meats under different conditions revealed the predominance of *Pseudomonas* except in refrigerator meats, whereas *Acinetobacter* (38.2%) dominated over *Pseudomonas* (32.7%) (Table 3). Eribo and Jay (1985) and Ayres (1960 b) observed similar result. It was also observed that *Acinetobacter* was the second important

TABLE 3. PERCENTAGE OF BACTERIAL ISOLATES FROM MEAT IN DIFFERENT CONDITIONS (cfu × 10^8/g)**

Total isolates	*Acinetobacter* spp. TVC	*Acinetobacter* spp. %	*Moraxella* spp. TVC	*Moraxella* spp. %	*Pseudomonas* spp. TVC	*Pseudomonas* spp. %
Fresh meat						
99.89	31.65	31.68	24.55	24.57	43.69	43.68
Spoiled meat (Room Temperature)						
5859	1085	18.50	2260	38.57	2514	42.90
Refrigerated meat (4°C for 10 days)						
113.77	43.50	38.23	33.06	29.05	37.23	32.72
Freezer meat (-20°C for 20 days)						
49.56	5.150	10.39	2.78	5.60	41.63	83.99

**= This result is the average of three experiments

bacterium isolated from meat but in spoiled meat, *Moraxella* (38.57%) dominated over *Acinetobacter* (18.55%) (Table 3). The development of spoilage flora and the shelf life of meat (goat and sheep) at room temperatures (26 to 32°C) were studied. There was an initial lag phase of 6 h before the bacterial growth was initiated. The meats were not cold initially, their temperatures varying from 34 to 37°C. There was no warming up period during the study and the humidity range was between 61 and 76%. Meat samples were covered by 0.2 mm pore size mesh and were allowed to proper aeration but strictly protected from entrance of any fly. There was surface drying of the meats up to 6 h. The bacterial growth was hindered by such drying (Scott 1936). Hence, surface drying might be one

of the causes for the initial lag phase and slow growth of the bacteria on meats.

Development of off-odour was observed at 18 h in all the meat samples, when the bacterial counts reached eight log cycle (1.2×10^8) per gram of meat and the colour of the meats became dark red instead of red. So, the shelf life was 17 h. Similar observation has been reported by Narasimha Rao and Sreenivasamurthy (1985) and they observed that off-odour developed at 20 h in all the carcasses, when the microbial count reached 8.5 log/cm^2 and the carcasses had a shelf life of 19 h. Off-odours were evident by second or third day, when beef was held 25°C (Ayres 1955). In view of this, internationally five million aerobic counts/g were suggested as microbial limits for fresh meat (Carl 1975). The Government of India has prescribed microbiological standards for raw meat (chilled/frozen): the total aerobic counts should not exceed 10^6 to 10^7/g (The Gazette of India, January 1993; Thulasi 1997). Comparing to the above standards, the present study showed that it had exceeded all the limits and warned that the meats from retail market at Pune city, India are not hygienic to consumers.

Conclusion

It may be concluded that *Acinetobacter* was found to be the dominant flora in meats next to *Pseudomonas. Moraxella* also formed a major part of the final flora in meats. The shelf life of meat at ambient temperature was found to be 17 h. Holton's medium without cefsulodin was found to be the most satisfactory selective medium for the recovery of *Acinetobacter* from meat.

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RESEARCH PAPER

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Effect of Processing on Mancozeb Residues in Apple

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Simple decontamination processes like washing and peeling dislodged the residues of mancozeb (Dithane M-45) in apple fruits to the extent of 97%. Washing coupled with peeling gave more relief from mancozeb residues than washing or peeling alone. Among apple processed products, although juice did contain mancozeb residues (1.4 mg/kg CS_2 equivalent), the level was far below the tolerance limit (3 mg/kg CS_2 equivalent). Jam and wine were free from residues of this pesticide. Maximum resdues were found in the apple pomace (5-9 mg/kg CS_2).

Keywords : Apple, Porocessing, Mancozeb residues.

Ethylene bis-dithiocarbamates (EBDC) are used to control various fungal diseases of fruits and vegetables (Thakur 1987). Apple is vulnerable to the attack of fungal pathogens causing diseases, such as scab (*Venturia inaequalis*) and leaf fall. Six sprays of mancozeb as a protectant fungicide has been recommended for the control of these diseases (Anon 1989). In actual practice, mancozeb is being used extensively by apple growers. There is no prescribed MRL's for mancozeb on processed products of apple. It was therefore, important to gather information on this aspect following recommended practices for apple cultivation. Hence, the present studies were undertaken to determine the level of mancozeb residues in raw apple fruit and its processed products, following treatment of Dithane M-45.

Materials and Methods

Fruits and pesticides : Dithane M-45 containing 75% active ingredient of mancozeb (a mixture of 2.5% zinc and 20% manganese) was sprayed in a block of 'Golden Delicious' cultivar of apple located at the R.F.R.S., Mashobra, Dist.. Shimla (7200 f.a.m.s.l.). Concentrations of 0.25% and 0.5%, each replicated four times having 3 plants in each replication were sprayed to run-off by power sprayer. Two sprays were given close to harvest with 10-12 litres spray suspension/tree at an interval of 20 days. Identical portions of trees were maintained as controls and were sprayed with water.

Sampling/harvesting of fruits : After 4 h of second mancozeb spray, about 60 kg fruits were collected for residue analysis at a height of 1.5-2.0 m above ground and from the inside and outside regions of each tree. The samples collected from each tree were kept in labelled field crates.

Fruit processing : Fruits from all the replicates were combined into a composite sample of two to three field crates per treatment for simple decontamination and processing studies. Apples were kept at ambient temperature and processed within 24 h of treatment. On the first day of harvesting, 1/3 portions of fruits from each composite lot of treatment were stored in boxes at ambient temperature for further analysis. The remaining fruits of each treatment were used for processing.

Sampling : For residue analysis in raw fruits, four apples were drawn randomly from each treatment alongwith the control. Each analysis was carried out in triplicate. Washing was done in cold, flowing tap water for 5 min, then drained for 2-4 min. Pulp samples were obtained by removing the peel (1mm thickness). The residues in whole and peeled fruit samples were determined simultaneously at different time intervals over a period of 20 days. For residue estimation, apples were chopped and 100 g representative sample drawn randomly from a mixed lot was used for further analysis.

Processed products : For processing, the fruits of each treatment were first washed in cold water, flowing tap water and then drained. Washed apples were cored, peeled and sliced manually with stainless steel knives. The fruit pieces were steamed for 5-6 min. The cooked slices were pulped in a tomato pulper with a 1.5 mm screen, as done commercially. The apple pulp was mixed with sugar (1:1) in a container and the whole mass was continuously cooked to the desired consistency (TSS 68 °Brix). The hot apple jam was filled in hot water-sterilized well-labelled wide-mouthed bottles and stored for further analysis (Lal et al. 1986). For juice preparation, washed apples of each treatment were grated in a stainless steel grater, followed by pressing in a cloth and rack press. Juice was collected in plastic containers. Half of the juice of each treatment was filled hot in hot-water sterilized glass bottles of 200 ml capacity, crown corked, pasteurized in boiling water for 15-20 min and immediately cooled. These were labelled and later used for residue analysis. The remaining juice was supplemented with sugar to 24°Brix, DAHP at 0.1% and KMS at 100 ppm and stored in big jars for inoculation with *Saccharomyces cerevisiae* at 5% of 24 h active culture to make wine as per the standard method (Joshi 1997). The fermentation was conducted at a temperature of 22 ±1°C. It took about 10 days for the completion of fermentation. It was allowed to mature further for another 5-6 months. During this period, the wines were siphoned, clarified and finally, bottled for residue analysis. The remaining portion after pressing the grated apple, was apple pomace. It was used for residue analysis as such.

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Residue analysis : The samples of raw fruits/processed products in each of the treatments were mixed and a sub-sample of 100 g was drawn for analysis of mancozeb, based on evolution of carbon disulfide (CS_2), resulting from acid hydrolysis and measuring spectrophotometrically (Dubey and Nath 1991). The level of residues in the samples was expressed as mg of carbon disulfide per kg raw fruit/product. The half-life in whole fruits was calculated as per the method of Hoskins (1961).

Results and Discussion

The mancozeb residues receded to more than 50% within 5 days of treatment in storage and on the 20th day, the amount was found to reach the tolerance level i.e. 3 mg/kg CS equivalent (Table 1). However, residues dissipated at relatively slow rates in fruits kept in storage as compared to the fruits on tree (Nath and Sharma 1993). It may be due to continuous growth of fruits on tree. The half-life of the stored fruits was found to be 13 days. Further, the rate of dissipation of mancozeb was much slower than that reported for tomato, potato and brinjal (Blazquez 1973; Rhodes 1977; Kumar and Agarwal 1990).

Efficacy of decontamination processes : The simple decontamination process like washing alone was effective in removing the mancozeb residues to a great extent i.e. 1.48-1.52 mg/kg CS_2, providing a relief of 73-83% (Table 2). But the mancozeb contents were found comparatively more i.e. 3.14-5.50 mg/kg in unwashed peeled fruits, which provided a relief of only 43% after 1 day of treatment. Washing followed by peeling removed the residues by more than 97% i.e. 0.15-0.20 mg/kg on 1st day of treatment. The effect of this simple decontamination process was found to be in decreasing trend with the passage of time, as on 20th day of treatment, the per cent relief provided by the washing, peeling and washing + peeling jointly reduced to 24, 25 and 56-60 %, respectively. The decrease in efficacy may be attributed to the fact that traces of mancozeb might have translocated to pulp with the passage of time. In an earlier study, plain washing removed 20-52% mancozeb residues and washing coupled with cooking led to 53-79% decontamination in fresh vegetables (Shrama et al. 1994).

Processing of fruits into juice, jam and wine resulted in a significant reduction of mancozeb residues (Table 3). There was a complete removal of residues from apple jam even after one day of treatment. But residues in apple juice increased slightly (1.10-1.47 mg/kg) over jam, showing about 37-49 % of the tolerance limit and providing relief of about 80-85%. It might be the result of technique of jam preparation where the pulp was cooked with equal quantity of sugar, thus reducing the relative concentration of the pesticide in the product, contrary to juice where such phenomenon did not occur. No residue of mancozeb could be detected in wine. Maximum residues (5.82-8.84 mg/kg) were, however, found in pomace and these were 176-295% of the tolerance limit. The occurrence of higher pesticide residues in pomace is on the expected lines, as the pesticide in the skin must have accumulated in the pomace. Maximum residues detected in pomace indicated that residues remained on the skin to about 1-2 mm thickness and this was

TABLE 1. EFFECT OF STORAGE ON MANCOZEB RESIDUES IN APPLE FRUITS

Days of storage	Residues, mg/kg	
	0.25 % (conc)	0.50 % (conc)
0	10.66	15.21
1	5.49 (42.81)	9.72 (36.09)
5	4.96 (53.17)	7.25 (52.33)
10	3.28 (69.23)	3.34 (78.04)
20	3.00 (71.86)	3.10 (79.62)-

Figures in parentheses are per cent degradation of mancozeb residues

TABLE 2. EFFECT OF SIMPLE DECONTAMINATION PROCESSES ON MANCOZEB RESIDUES IN APPLE FRUITS

Treatment	Days	Percent relief at concentrations	
		0.25 %	0.50 %
Peeled	1	42.81 (3.14)	43.42 (5.50)
	5	53.83 (2.29)	58.90 (2.98)
	10	39.63 (1.98)	29.64 (2.35)
	20	26.67 (2.20)	25.81 (2.30)
Washed	1	73.04 (1.48)	83.85 (1.57)
	5	74.19 (1.28)	82.41 (1.42)
	10	63.41 (1.20)	82.41 (1.25)
	20	25.00 (2.25)	24.19 (2.35)
Washed + peeled	1	97.27 (0.15)	97.99 (0.20)
	5	90.73 (0.46)	91.59 (0.61)
	10	72.87 (0.89)	72.46 (0.92)
	20	60.00 (1.20)	56.45 (1.35)

Figures in parentheses are mancozeb residues after processing

TABLE 3. PERCENT RELIEF FROM MANCOZEB RESIDUES IN PROCESSED APPLE FRUIT PRODUCTS

Product	Days	Percent relief at concentrations	
		0.25 %	0.50 %
Pomace	1	3.83 (5.28)	9.05 (8.84)
Juice	1	79.96 (5.28)	9.05 (8.84)
Jam	1	100.00 (0.00)	100.00 (0.00)
Wine	20	100.00 (0.00)	100.00 (0.00)

Figures in parentheses are mancozeb residues after processing

in confirmity with the results of decontamination processes like washing coupled with peeling. The data on the effect of mancozeb residues in processed products revealed that preparation of jam and pasteurization of apple juice at high temperature resulted in a significant reduction of mancozeb residues. Kolankaya et al (1989) reported that the level of mancozeb in potatoes was 0.66 mg after 3 months of storage for the applied dose of 1.0 mg, while the deep-frying at high temperature in sunflower oil decreased the amount to 0.072 mg. Similarly, when bean pods treated with mancozeb and weathered for 14 days were heated for 30 min. at high temperature in an autoclave (Musumeci et al. 1989), the mancozeb contents reduced only slightly from 4.6 to 4.2 mg/kg. Reduction in fungicide concentration in wine could be attributed to the process of degradation during fermentation or precipitation in the conditions prevailing in the fermentation or ageing. In

earlier studies, complete degradation of pesticides in grape wine preparation has also been reported (Flori and Cabras 1990).

Since the fungicides of concern to EPA are largely contact fungicides, it may be possible to develop methods, such as washing, peeling or temperature treatments to remove residues. In the present studies, mancozeb was at or below the detection limit in processed apple products, whereas it was below the tolerance limit in washed and peeled apples at different intervals. In general, such methods may be needed from consumer point of view, because the strategy of increasing the interval between last application and harvest to reduce risks of late season outbreaks of pre-and post-harvest diseases.

Conclusion

This study has revealed that the amount of mancozeb that can penetrate into the pulp might remain in the first few millimeters under the peel. Following sprays even 4 h before harvest on apple fruits, the residue values obtained were lower than those acceptable limits (3 ppm) by the World Health Organisation (Parmar et al. 1988). From the consumption of fresh fruits and processing points of view, the simple decontamination processes like peeling and washing assume considerable significance. On the basis of residue data generated in apple products viz. jam and wine, an MRL of "0" ppm can be recommended. This will help in assessing the residue levels in such products sold to the consumers.

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RESEARCH PAPER

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Preservation of Pineapple, Mango and Papaya Chunks by Hurdle Technology

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Storage life of pineapple, mango and papaya fruit chunks preserved by hurdle technology was studied using the hurdles pH, mild heat treatment preservatives and packaging. Pineapple and mango fruit chunks, blanched in syrup at 85°C for 5 min. dipped in syrup containing 340 mg of potassium metabisulphite/kg and 413 mg sodium benzoate/kg for 8 h and packed in 150 gauge polypropylene pouches showed acceptable sensory and microbiological qualities up to a period of 30 days at 27°C and 60 days at 2°C and had SO_2 content of 65-85 mg/kg and benzoic acid 150-200 mg/kg. The papaya chunks treated with increases in levels of preservatives to 680 mg potassium metabisulphite/kg and 826 mg sodium benzoate/kg exhibited good storage stability up to 90 days at 2°C and ambient temperature.

Keywords : Fruit chunks, Hurdle technology, Preservation, Mango, Papaya, Pineapple..

Shelf stability of minimally processed products can be enhanced by using a combination of treatments such as blanching, osmo-dehydration, addition of chemical preservatives, modification of pH and appropriate packaging (Huxsoll and Bolin 1987). This technology is known as Hurdle Technology. The different treatments called as hurdles are used in preventing the chemical as well as microbiological deterioration of the food product. A combination of these hurdles is very effective, while individually each hurdle is ineffective in protecting food from deterioration (Leistner 1994).

Hurdle technology has also been used for the preservation of minimally processed fruit slices. A combination of mild heat treatment, reduction of water activity, lowering of pH and addition of potassium sorbate and sodium bisulphite for producing shelf stable pineapple slices was reported by Alzamora et al (1989). High moisture products from peach, pineapple, mango, papaya, chicozapote and banana treated with a combination of mild heat treatment, water activity reduction, addition of antimicrobials and packed in glass flasks or high density polyethylene bags with syrup containing preservatives, had a shelf stability ranging from 4 to 8 months (Alzamora et al. 1993). They observed that pineapple slices blanched in saturated vapour for 2 min, cooled in water and immersed in a glucose syrup containing 150 mg/kg sodium bisulphite and 1000 mg/kg potassium sorbate were acceptable up to 120 days of storage.

However, in all these studies, the fruit chunks were stored in syrup containing high concentrations of preservatives. The usage of covering syrup not only increases the bulk but also increases the cost of the product. Therefore, there is a need to explore the possibility of preserving fruit chunks of tropical fruits such as pineapple, mango and papaya in a cost effective method. The main objective of the present investigation was to study the effect of different barriers or hurdles viz. osmo-blanching, reduction of pH, addition of preservative and vacuum packaging on the shelf stability of fruit chunks of pineapple, mango and papaya packed in polypropylene pouches, without covering syrup.

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Materials and Methods

Fruit chunks : Mature, firm ripe pineapples, mangoes (C.V. 'Neelum'), and papayas were procured from the local fruit market, washed, manually peeled, cored and cut into 1 inch × 1 inch × 0.5 inch dimensions. The cut fruit chunks were used for different treatments. 20°Brix [a] and 40°Brix [b] sucrose syrup with 0.2% citric acid at 85° C for 5 min using 1:2 fruits to syrup ratio. The blanched fruit chunks were further dipped in 20 °Brix syrup containing 0.2% citric acid, 340 mg potassium metabisulphite/kg and 413 mg sodium benzoate/kg for 8 h at ambient temperature, drained and packed in 150 gauge polypropylene pouches and sealed;

The pineapple and mango chunks stored at 2°C and 27°C were anlysed at 0, 30 and 60 days for the chemical, microbiological and sensory qualities.

The papaya fruit chunks were blanched in 40°Brix syrup containing 0.6% citric acid at 85°C for 5 min. The blanched fruit chunks were then dipped in 40°Brix syrup at 27°C containing 0.6% citric acid, 680 mg potassium metabisulphite/kg and 826 mg sodium benzoate/kg for 8 h. The chunks were drained and packed in polypropylene pouches with and without vacuum, using vacuum sealing machine (KOMET, Germany). The pouches were withdrawn at 30, 45, 60 and 90 days storage and analyzed for chemical and microbiological qualities.

Chemical analysis : Moisture, total solids, reducing sugars, total sugars and carotenoids were analyzed as per the procedure described by Ranganna (1986). pH was measured using a digital pH meter, Model No. APX 175, Control Dynamics, Bangalore, India. Sulphur dioxide content of the sample was distilled in the presence of acid in an inert atmosphere and absorbed by Iodine, which converts sulphurous acid to sulphuric acid and titrated against standard sodium thiosulphate solution. The SO_2 was reported as free and combined (Askar and Treptow 1993). The sample was saturated with NaCl solution and neutralized by excess NaOH. The benzoic acid

present in the sample was converted into water soluble sodium benzoate, the solution was acidified with excess HCl, water insoluble benzoic acid was formed, and extracted with chloroform. The chloroform was removed by evaporation and the residue containing benzoic acid was dissolved in alcohol and titrated against standard NaOH (Askar and Treptow 1993).

Nutrient retention studies : The kinetic of nutrient in papaya fruit chunks during storage was determined by using a first order reaction model.

$A = A_o e^{-kt}$

where, A = Nutrient value at any given time 't',

A_o = Initial nutrient value and

K = Reaction rate constant

Sensory evaluation : The samples were assessed for colour, flavour, texture and overall quality by a 15 member trained panel on a 10 point scale, where 1-2 = poor, 3-4 = fair, 5-6 = good, 7-8 = very good, and 9-10 = excellent (Askar and Treptow 1993). Samples receiving an overall quality score of 7 or above were considered acceptable and those receiving below 7 were considered unacceptable. The sensory data were subjected to two way analysis of variance and the difference between the means was analyzed by Duncans multiple range test (Snedecor and Cochran 1967).

Mesophilic aerobes, yeasts and molds were enumerated using plate count and coliform numbers by the most probable number technique (Speck 1984).

Results and Discussion

The pHs of mango and papaya fruit chunks got reduced to below 4.0 after the syrup treatment as compared to the pH of fresh chunks (Table 1). The reduction of pH and penetration of sugar into the treated fruit chunks also improved the sensory quality by establishing sugar acid ratio in addition to the preservative effect. King and Bolin (1989) also observed that reduction of pH in case of fruits having a pH of 4.5 and above also functioned as an effective hurdle in improving the shelf stability of minimally processed products. Lower pH caused unfolding of the protein molecule by disruption of the hydrogen bonds, thereby reducing the enzyme activity and increasing its sensitivity to denaturation. There was no significant change in pH in all the treatments during storage. The SO_2 concentration in the treated pineapple and mango chunks ranged from 65 mg/kg to 80 mg/kg and the benzoic acid from 150 mg/kg to 200 mg/kg. The treated papaya chunks had higher amount of SO_2 (135 mg/kg) and benzoic acid (135 mg/kg).

In case of papaya where initial total soluble solids content was very low i.e. 10 °Brix the syrup treatment improved colour, flavour and acceptability. The pH of the fruit slices adjusted to 3.5-3.8 had not effected the natural taste of the product.

Effect of storage on chemical changes in pineapple chunks: Reducing sugars of the pineapple chunks showed a steady increase during storage in both [a] and [b] treatments after 30 days and 60 days storage and the increase was found to be maximum in the samples stored at 27°C (Table 2). This could be explained by the higher rate of inversion under acidic conditions at high temperature. There were no significant differences in total sugars in both the treatments stored at 2°C and 27°C. The retention of total carotenoids was found to be maximum in the samples stored at 2°C, indicating the adverse effect of temperature on the samples stored at 27°C. A marginal increase in total solids was observed in 60 days storage of both the treated samples, which could be due to the loss of moisture during storage.

Effect of storage on chemical changes in mango chunks: Reducing sugar contents of mango chunks stored at 27°C increased during storage. Total carotenoid contents decreased in the mango chunks stored at 27°C, whereas, there were no significant changes in the samples stored at 2°C, indicating better storage stability at low temperature. The changes in total sugars were not appreciable. Total solids content increased marginally during storage, indicating loss of moisture (Table 2).

Effect of storage on microbiological quality of pineapple chunks : Pineapple chunks subjected to the blanching treatment of [a] and stored for 30 and 60 days showed mesophilic aerobic counts of 640 and 1000 cfu/g, respectively. In case of chunks subjected to treatment [b], the count was 500 cfu/g both at 30 and 60 days of storage at 27°C. There were significant

TABLE 1. pH AND PRESERVATIVE LEVELS IN TREATED FRUIT CHUNKS

Treatment	pH		SO_2 mg/kg	Benzoic acid, mg/kg
	Fresh	Treated		
Pineapple chunks				
[a]. Dipped in 20°Brix syrup containing 0.2% CA, 340 mg potassium metabisulphite/kg and 413 mg sodium benzoate/kg	3.56	3.35	74.89 ± 1.82	174.6 ± 0.95
[b]. Dipped in 40°Brix syrup containing 0.2% CA, 340 mg potassium metabisulphite/kg and 413 mg sodium benzoate/kg	3.56	3.36	80.68 ± 1.04	200 ± 1.70
Mango chunks				
[a]. Dipped in 20°Brix syrup containing 0.2% CA, 340 mg potassium metabisulphite/kg and 413 mg sodium benzoate/kg	4.43	3.67	65 ± 1.70	150 ± 1.29
[b]. Dipped in 40°Brix syrup containing 0.2% CA, 340 mg potassium metabisulphite/kg and 413 mg sodium benzoate/kg	4.43	3.78	67 ± 1.41	180 ± 1.26
Papaya chunks				
[c] Dipped in 40°Brix syrup with 0.6% CA, 680 mg potassium metabisulphite/kg and 826 mg sodium benzoate/kg	5.55	3.60	135 ± 1.30	320 ± 1.29

Mean ± S.D, n = 4

TABLE 2. CHEMICAL COMPOSITION OF PINEAPPLE AND MANGO CHUNKS DURING STORAGE

Storage period	Moisture, g/100 g	Total solids, g/100g	Reducing sugars, g/100g	Total sugars[1], g/100g	Total carotenes µg/100g
Pineapple					
0 day[1] [a]	77.55	22.45	3.74	17.55	261
0 day[1] [b]	62.00	38.00	4.55	30.00	195
30 days					
[a] at 2°C	77.46	22.54	3.75	17.59	245
[a] at 27°C	77.00	23.00	5.54	18.45	141
[b] at 2°C	61.68	38.32	4.57	30.05	180
[b] at 27°C	60.51	39.49	7.39	30.45	115
60 days					
[a] at 2°C	77.24	22.76	3.83	18.39	100
[a] at 27°C	76.77	23.23	6.23	17.65	23
[b] at 2°C	60.29	39.71	4.63	30.07	78
[b] at 27°C	59.80	40.20	8.85	29.37	19
Mango					
0 day[1] [a]	75.00	25.00	5.50	18.85	2350
0 day[1] [b]	63.72	36.28	8.29	29.49	2358
30 days					
[a] at 2°C	74.81	25.19	5.53	18.88	2022
[a] at 27°C	74.14	25.86	6.31	19.63	1982
[b] at 2°C	63.57	36.43	8.33	29.52	2247
[b] at 27°C	60.45	39.55	9.31	30.19	1127
60 days					
[a] at 2°C	73.58	26.42	5.57	18.90	2308
[a] at 27°C	73.30	26.70	6.40	19.01	1908
[b] at 2°C	63.00	37.00	8.40	29.60	1007
[b] at 27°C	62.00	38.00	10.04	30.00	738

The initial values for total sugars in fruit chunks blanched and treated in sugar syrup increased with the strength of syrup used, as the cane sugar penetrated the diced chunks.
[a] treated with 20° Brix syrup [b] treated with 40° Brix syrup

TABLE 3. MICROBIOLOGICAL QUALITY OF PINEAPPLE AND MANGO CHUNKS DURING STORAGE

Storage period	Aerobic count, cfu/g	Yeast and mold, cfu/g
Pineapple		
0 day[1] [a]	5	0
0 day[1] [b]	5	0
30 days		
[a] at 2°C	80	10
[a] at 27°C	640	10
[b] at 2°C	60	10
[b] at 27°C	500	10
60 days		
[a] at 2°C	500	3000
[a] at 27°C	1000	3500
[b] at 2°C	500	3000
[b] at 27°C	500	3500
Mango		
0 day[1] [a]	10	Nil
0 day[1] [b]	5	Nil
30 days		
[a] at 2°C	40	40
[a] at 27°C	80	80
[b] at 2°C	40	40
[b] at 27°C	60	60
60 days		
[a] at 2°C	200	200
[a] at 27°C	800	800
[b] at 2°C	200	200
[b] at 27°C	800	800

Microbial quality immediately after the syrup treatment and packaging of chunks.

[a] chunks treated with 20° Brix syrup; [b] chunks treated with 40°Brix syrup

increases in yeast and mold counts after 60 days of storage in both [a] and [b] treatments (Table 3). The pineapple chunks had an acceptable quality up to 30 days storage at 27°C. Coliform count was found to be negative in all the treatments of pineapple chunks during the entire period of storage. Alzamora et al (1989) also reported that pineapple slices preserved by combined methods and stored for 4 months at 27°C exhibited no growth of microrganisms during 30 days storage. The lower microbial growth rates in their study could be due to the higher level of preservatives used and packing the fruit slices along with the syrup containing preservatives.

Effect of storage on microbiological quality of mango chunks : Mango chunks subjected to treatments [a] and [b] and stored for 30 days at 27°C had aerobic counts of 80 and 60, respectively. The aerobic counts increased to 800 in both the treatments after 60 days storage (Table 3). Yeast and mold count was 40 at 30 days storage and it increased significantly after 60 days storage in all the treatments. Coliform count was negative in all the treatments.

Effect of storage on sensory quality of pineapple chunks: Two-way analysis of variance showed significant difference in overall quality of all the treatments at 5% level. The pineapple chunks with treatment [a] and [b] and stored for 30 days at 2°C showed higher overall quality. Pineapple chunks treated with 40°Brix sugar syrup [b] showed better sensory acceptability than those treated with 20° Brix, at 27°C for 30 days storage. However, the overall quality scores reduced significantly at 60 days of storage. (Table 4). The pineapple chunks had bleached appearance after 60 days of storage. Alzamora et al (1989) also reported that pineapple slices preserved by combined methods showed discoloration after 83 days of storage, rendering the product slightly whiter than the original fruit.

Effect of storage on sensory quality of mango chunks : Sensory analysis of the mango chunks during storage indicated that the product was highly acceptable up to 30 days at 27°C and 60 days at 2°C. Two way analysis of variance indicated significant difference ($p \leq 0.05$) in overall quality in all the treatments. The mean scores of the samples stored at 2°C were superior and significantly different from the samples stored at 27°C (Table 4). It can be inferred from the above studies that the pineapple and mango fruit chunks treated with [b] i.e. 40 °Brix sugar syrup had superior quality as compared to [a] i.e. 20 °Brix sugar syrup.

TABLE 4. SENSORY QUALITIES OF PINEAPPLE AND MANGO CHUNKS DURING STORAGE

Treatment	Mean overall quality scores	
	Pineapple	Mango
0 day	8.15	8.20
30 days		
[a] Treated with 20°B and stored at 2°C	8.13[a]	8.17[a]
[a] Treated with 20°B and stored at 27°C	7.37[b]	7.49[b]
[b] Treated with 40°B and stored at 2°C	8.22[a]	8.50[c]
[b] Treated with 40°B and stored at 27°C	7.74[c]	8.08[a]
60 days		
[a] Treated with 20°B and stored at 2°C	7.03[a]	7.44[c]
[a] Treated with 20°B and stored at 27°C	6.07[b]	6.48[a]
[b] Treated with 40°B and stored at 2°C	7.50[c]	7.66[b]
[b] Treated with 40°B and stored at 27°C	6.44[d]	6.33[a]

*Means with different superscripts are significantly different at ($p \geq 0.01$)

TABLE 5. REACTION RATE CONSTANTS AND R^2 VALUES OF PACKED PAPAYA CHUNKS DURING STORAGE AT 2°C AND 27°C

Treatment		
Reducing Sugars		
2°C (VP)	0.0002	0.93
2°C (OP)	0.0002	0.90
27°C (VP)	0.0043	0.85
27°C (OP)	0.0045	0.90
Carotenes		
2°C (VP)	-0.0002	0.894
2°C (OP)	-0.0004	0.930
27°C (VP)	-0.0002	0.900
27°C (OP)	-0.0005	0.950

* Obtained by linear regression of nutrient content and storage period
VP - Vacuum packed; OP - Ordinary packed

Reducing sugars of papaya chunks stored at 27°C showed a similar trend of increase as during storage at 27°C whereas, there was no significant change in total sugars. The retention of carotenoids was maximum (93%) at 30 days storage in all the samples. The carotenoids showed slight reduction in both the vacuum packed and without vaccum packed samples at 45 days and 60 days storage. Maximum losses of carotenoids (25%) were observed at 90 days storage at 27°C.

Kinetics of reducing sugars and carotenes in packed papaya chunks during storage : Fig. 1 and 2 show the kinetics of reducing sugars and carotenes during storage of packed papaya chunks at 2°C and 27°C. Reducing sugar contents of packed papaya chunks increased with increase in temperature and storage period. The reaction rate constant (k) was higher at higher storage temperature (27°C), indicating inversion of sucrose into reducing sugars in presence of citric acid. Lower reaction rates were observed for the papaya chunks stored at 2°C (Table 5). The increase in reducing sugars during storage of mango ready-to-serve beverage was also reported by Beerh et al (1989).

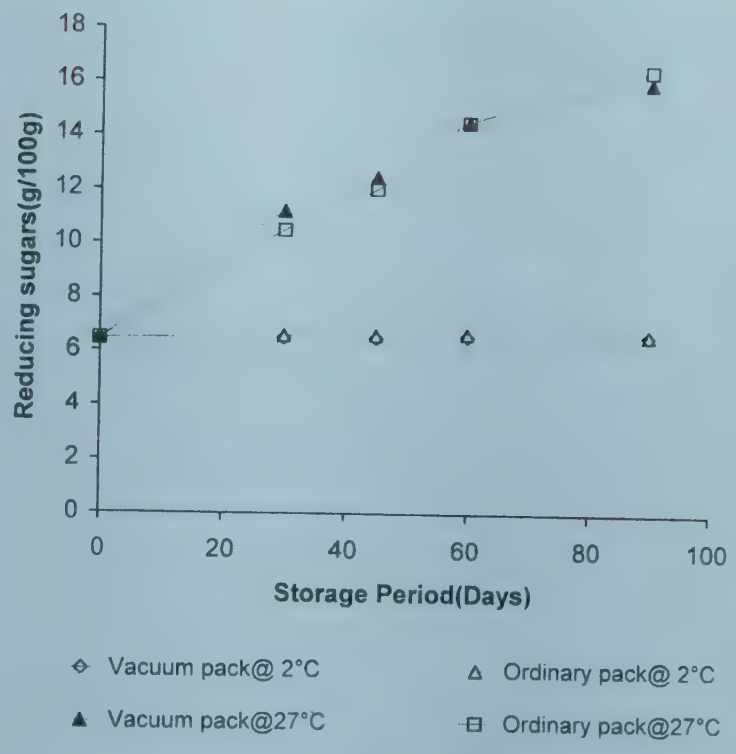

Fig. 1. Kinetics of Reducing sugar in papaya chunks during storage

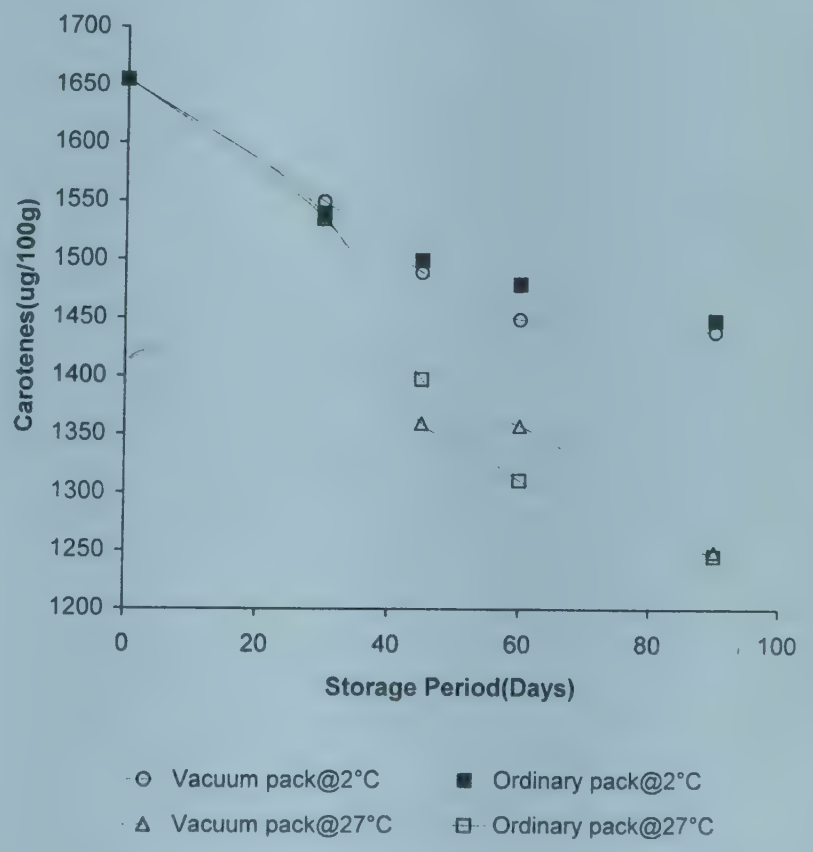

Fig. 2. Kinetics of Carotenes in papaya chunks during storage

Carotene contents of papaya chunks decreased with increase in temperature (27°C) during storage. It was a first order reaction rate constant 'k'. The reaction rate (k) was higher at higher temperature, indicating faster degradation of carotenes at 27°C than at 2°C. Also, the product packed under vacuum had lower degradation of carotenes, as compared to ordinary packing. This might be due to the fact, that carotene was more sensitive to oxygen in the storage atmosphere (Fig. 2).

These studies have indicated the effect of storage conditions in particular temperature and the method of packing, on

TABLE 6. AEROBIC-MESOPHILIC AND YEAST AND MOLD COUNTS OF PAPAYA CHUNKS DURING STORAGE

Storage period	VP 2°C	VP 27°C	OP 2°C	OP 27°C
Aerobic count				
0 day	4	4	4	4
30 days	300	500	400	600
45 days	400	500	400	600
60 days	800	1000	1200	1200
90 days	180	180	150	220
Yeast and Mold count				
0 day	Nil	Nil	Nil	Nil
30 days	10	10	10	10
45 days	10	10	10	10
60 days	20	20	20	20
90 days	10	10	10	10

*VP - Vacuum packed; OP - Ordinary packed

TABLE 7. SENSORY QUALITIES OF PAPAYA CHUNKS DURING STORAGE

Storage period	Overall quality of papaya chunks *			
	VP 2°C	VP 27°C	OP 2°C	OP 27°C
Aerobic count				
0 day	8.00^{a}	8.01^{a}	7.98^{a}	8.00^{a}
30 days	7.35^{a}	7.24^{a}	7.65^{b}	7.60^{b}
45 days	7.32^{a}	7.30^{a}	7.50^{b}	7.37^{a}
60 days	7.20^{b}	7.10^{b}	7.51^{a}	7.20^{b}
90 days	6.60^{a}	6.50^{a}	7.25^{b}	7.00^{c}

*Means with the same superscript are not significantly different at $p \geq 0.01$
*VP - Vacuum packed; OP - Ordinary packed

the changes in the reducing sugars and carotenes during storage. By determining the 'k' value, the rate at which these quality parameters deteriorate over the storage period can be evaluated. To retain the maximum nutrients, appropriate conditions can be applied.

Effect of storage on microbial quality of papaya chunks: Microbial study during 3 months storage at 27°C indicated no significant growth of microorganisms, indicating the stability of the product during storage. The aerobic counts increased marginally at 60 days storage and decreased at 90 days storage. There was no growth of yeast and mold and coliform count was found to be negative during the entire period of storage (Table 6). Guerrero et al (1994) reported that aerobic plate count increased by two logarithmic cycles and then decreased, reaching values similar to initial one during storage of banana *puree* by combined methods.

Effect of storage on sensory quality of papaya chunks : Table 7 shows the overall quality of papaya chunks during storage. Two way ANOVA indicated significant differences among the treatments at the end of 30 days storage. The overall quality of papaya chunks packed without vacuum was significantly superior to other treatments. The vacuum-packed papaya had slightly lower overall quality scores as compared to papaya chunks packed without vacuum because of leaching of juice and partial softening of the tissue during vacuum packing. The papaya chunks packed without vacuum and stored at 2°C had superior overall quality, followed by the papaya chunks stored at 27°C. The overall quality ratings reduced at the end of 90 days storage but the papaya chunks were still acceptable.

Lopez-Mayo et al (1994) reported blanched papaya slices equilibrated in syrup for 3 days with continuous circulation of syrup packed along with the syrup containing, pH 3.5, 1000 mg potassium sorbate/kg and 150 mg sodium bisulphite/kg in the glass jars had a storage life of 5 months at 25°C. The longer storage life in their study was attributed to the higher level of preservatives and packing of slices along with the syrup.

In all the earlier reported studies involving the preservation of fruit slices by combined methods, the fruit slices were packed with syrup containing preservative either in glass flasks or high density polyethylene pouches. The fruit slices were also blanched with saturated steam before the treatment. However in the present study, the fruit chunks were blanched in sugar syrup, followed by a dip in syrup containing preservatives. The significance of blanching in sugar syrup was to inactivate the enzymes, reduce microbial load, as well as partial reduction in water activity due to osmotic dehydration during syrup treatment (Vijayanad et al. 1993). The flavour of the fruit chunks can also be retained during osmo blanching as compared to the steam blanching where volatile flavour compounds may be lost. The preservative levels in the treated fruit chunks are also comparatively lower than those reported in earlier studies (Alzamora et al 1989, 1993; Guerrero et al. 1994).

It may be concluded that pineapple and mango fruit chunks, blanched in syrup at 85°C for 5 min, dipped in syrup containing 340 mg potassium metabisulphite/kg and 413 mg sodium benzoate/kg for 8 h and packed in 150 gauge polypropylene pouches have a shelf life of up to a period of 30 days at 27°C and 60 days at 2°C, whereas the papaya chunks treated with increase in level of preservatives 680 mg potassium metabisulphite/kg and 826 mg sodium benzoate/kg have exhibited good storage stability up to 90 days at 2°C and ambient temperature.

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RESEARCH PAPER

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Development of Antisera to Testicular Antigens for Identification of Species Origin of Meats Using DID, IE, CIE and RIE Techniques

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Antisera were raised in rabbits using native and heated testicular antigens from cattle, buffalo, sheep and goat. Double immunodiffusion test (DID), Immunoelectrophoresis (IE), counter-immunoelectrophoresis (CIE) and rocket immunoelectrophoresis (RIE) techniques were standardized using the above sera. Cross reactions were noticed between different species antigens and they were eliminated by making the sera monospecific, using glutaraldehyde treated immunosorbent of the heterologous antigens. To overcome the problem of absorption, to make the sera monospecific, antisera were also raised in phylogenetically related species, viz., cattle in buffalo, buffalo in cattle, sheep in goat and goat in sheep using native as well as heated testicular antigens which proved monospecific directly. The sera samples could detect adulteration in fresh meat samples even, at a low level, of 1%, while in the case of cooked meat samples, the detection levels were 10 to 20%. The sera were lyophilised and could show specific reaction up to 2 years.

Keywords : Antisera, Double immunodiffusion, Immunoelectrophoresis, Counter immunoelectrophoresis, Rocket immunoelectrophoresis, Testicular antigens, Monospecific, Adulteration.

In the quest for developing species-specific antibodies, Milgrom et al (1965) found heat stable antigens in boiled, ethanol precipated and saline extracts of adrenal gland, pituitary and testes. The other proteins reported to retain antigenic competence after heating were gamma globulins (Karpas 1970), myoglobin (Hayden 1979) and unheated chicken or turkey troponin (Hayden 1977). Though earlier studies based on these findings were inconclusive, some studies on adrenal gland (Hayden 1981; Reddy et al. 1990; Sherikar et al 1993) troponin (Saisekhar and Reddy 1995) and muscle antigens (Reddy and Reddy 1995) were proved to be promising. The present investigation was aimed to develop (i) species-specific antibodies against heated as well as fresh testicular antigens of cattle, buffalo, sheep and goat; (ii) to identify the origin of species of cooked as well as fresh meats; (iii) to compare and evaluate the suitability of developing antisera in rabbits as well as in phylogenetically related species against heated and fresh testicular antigens and (iv) to ascertain the validity of different tests like DID, IE, CIE and RIE.

Materials and Methods

Preparation of testicular antigens : The procedure of Milgrom and Witebsky (1962) was followed with slight modifications for the preparation of testicular antigens either fresh or heated (Fig. 1).

Muscle antigens : Native and heated antigens of muscle were prepared by obtaining 30 muscle samples from each species of cattle, buffalo. sheep, goat, pig and chicken.

Adulterated meat antigens : The 20% adulterated meat extracts of fresh and cooked at 120°C for 20 min were prepared by mixing beef with cara beef/mutton/chevon/pork/chicken at different levels.

Estimation of protein concentration : The protein concentrations in testicular antigens, and meat extracts of different species of animals were estimated as per the procedure of Lowry et al (1951) using Spectronic-21. (Baush & Lomb).

1	20 g testicular tissue + 100 ml 0.14 M NaCl sol. ↓	Homogenized, 10min Centrifuged 2000 rpm. 30 min.
2	Supernatant (Native saline extract)	Heated at 100°C, at for 1 h, homogenized and centrifuged at 3000 rpm. 15 min
3	Supernatant ↓	Autoclaved, ½ h, cetrifuged 2000 rpm. 30 min.
4	Supernatatnt + 3 Vol. of 71% ethanol	Kept at room temp. 12 h centrifuged at 2000 rpm. 15 min.
5	Precipitate dissolved In 20 ml of 0.14M NaCl sol ↓	Centrifuged at 15000 rpm. ½ h, to remove undissolved particles
6	Supernatant + 3 vol. of 71% ethanol ↓	As in step 4
7	Steps 4 and 5 Repeated thrice ↓	
8	Precipitate ↓	Dried at room temp. ground to fine powder
9	Metallic grey powder	Called BE (boiling resistant and ethanol precipitate

Fig. 1. Preparation of testicular antigens. Reddy et al (1990)

Antibody development in rabbits : The modified procedure of Milgrom et al (1965) was followed for immunization of rabbits against native as well as heated testicular antigens of cattle, buffalo, sheep and goat.

Cross immunization : Antibodies were raised in cattle, buffalo, sheep and goat against buffalo, cattle, goat and sheep

testicular antigens of either fresh or heated antigens and the sera thus developed were stored at -20°C in 1 ml aliquot samples in tightly closed screw cap bottles, after adding thiomersal as preservative.

Concentration of antibodies : The antisera harvested were concentrated by reducing the volume of moisture in the serum against 10 % polyethylene glycol, until the volume reached to its half the original quantity and also by ammonium sulphate precipitation (Campbell 1970).

Development of species - specific antibodies : For the absorption experiments, the method described by Reddy et al (1990) was followed to eliminate cross reacting antibodies by trial and error method of mixing the antibody with antigen and testing by DID to know its species specificity. The other method used for absorption of cross reacting antibodies was that Talwar (1983) in which glutaraldehyde treated immuno-sorbent was developed to make the sera monospecific.

Tests : Agar gel double immunodiffusion, Immunoelectrophoresis, counter immunoelectrophoresis and rocket immunoelectrophoresis techniques were used for identification of species origin of meats.

Freeze-drying : All the species-specific antisera were lyophilised in 1 ml aliquot samples and were used after reconstitution with 1 ml of normal saline solution.

Single radial Immunodiffusion (SRID) : This method was used to make accurate quantitative determination of the antigens. Standards of known concentrations and samples to be estimated were loaded in wells punched out in a gel containing monospecific antiserum raised against the antigen of interest. The area of the circular precipitates, developed by samples to be tested showed a linear relationship with the concentrations of the standards. The concentrations of the samples could be directly read off from the standard curves using the area obtained with standards (mancini 1965).

Results and Discussion

The results obtained with different antisera are presented in Table 1.

The rabbit anti cattle, native (RAC (N) testicular serum cross reacted with buffalo, sheep and goat muscle extract native antigens, indicating the presence of three antigenic fractions in DID. Out of the three antigenic fractions, (Plate 1) two were completely identical with buffalo, which maintained partial identity with sheep and goat muscle extract native antigens. The third antigenic fraction was distinct and species-specific. However, in IE (Plate 2) and CIE, it showed the presence of four antigenic fractions. The complete fusion of lines formed with cattle meat extract native (CME (N) and buffalo meat extract native (BME (N) in DID indicated the presence of identical antigenic determinants and with native sheep and goat meat extracts (SME N) and GME (N), it developed partial identity. The cross reacting antibodies in RAC (N) serum against native antigens, however, could be completely eliminated on adsorption and the serum could be made monospecific. The absorbed serum formed one precipitation line only with homologous native cattle muscle extract in DID,

TABLE 1. CONCLUSION ON DIFFERENT ANTISERA IN A NUT SHELL

Name of the antiserum	Reaction	Absorption	Detection of adulteration	
			Cooked meat	Fresh meat
RAC (N)	Cross reactions with buffalo, sheep and goat	Required	20%	10%
RAB (N)	Cross reactions with cattle, sheep and goat	Required	20%	10%
RAS (N)	Cross reactions with cattle, buffalo and goat	Required	20%	10%
RAG (N)	Cross reactions with cattle, buffalo and sheep	Required	-	10%
BAC (N)	No cross reactions; by itself monospecific	Not required	20%	10%
CAB (N)	No cross reactions; by itself species specific	Not required	20%	10%
GAS (N)	No cross reactions; by itself species specific	Not required	20%	10%
SAG (N)	No cross reactions; by itself species specific	Not required	20%	10%
RAC (BE)	Cross reactions with buffallo, sheep and goat	Required	5%	10%
RAB (BE)	Cross reactions with cattle, sheep and goat	Required	10%	20%
RAS (BE)	No cross reactions with cooked antigens; cross reactions with goat native antigens	Required	10%	20%
RAG (BE)	Cross reactions with sheep only	Required	10%	20%
BAC (BE)	No cross reactions with cooked antigens. Cross reaction with native buffalo	Not required	10%	20%
CAB (BE)	No cross reactions by itself species specific	Not required	10%	20%
GAS (BE)	No cross reactions; by itself species specific	Not required	20%	20%
SAG (BE)	No cross reactions; by itself specific specific	Not required	20%	20%

IE (Plate 3) and CIE (Plate 4), but there was no reaction with other muscle extracts under study. This absorbed serum could detect minimum levels of 10% and 20% fresh and cooked meats, respectively when adulterated with other species meats. The same RAC (N), serum showed the presence of single antigenic fraction, when tested with other homologous (CME (H) heated muscle extract and found to contain no antibodies against heated buffalo, sheep and goat muscle extracts. This indicated that the testicular antigens not only retained the antigenicity even after heat treatment but also species specificity or acquired new species specificity as observed by Hirose and

Osler (1967) and Henny and Ishizaka (1968). The rabbit anti buffalo, native (RAB (N) testicular serum also gave cross reactions. However, it proved to be species-specific after absorption only with homologous cara beef extract by giving single precipitation line either in DID or CIE. The serum showed the reactions of complete identity with cattle, indicating the presence of identical antigenic determinants in cattle and buffalo muscle extracts. The absorbed RAB (N) serum detected fresh cara beef to the lowest level of 10% when adulterated with beef/mutton/chevon. However the same serum even without absorption specifically identified cooked cara beef to a minimum level of 20% when adulterated with other meats giving a single line of precipitation in all the experiments (Plate 5).

Two out of the three antigenic fractions seen in SME (N) against rabbit anti sheep native (RAS (N) testicular antiserum (Fig. 2) were completely identical with CME (N). However, these two antigenic fractions indicated the presence of multispecifc antigenicity in SME (N), as they developed clear spurs on the arcs of interference of both the lines developed with GME (N) and BME (N) antigens. The third antigenic fraction which is species specific proved by itself when the RAS (N) serum gave reaction with cooked homologus (SME (H) only and no reaction with GME (H), CME (H) and BME (H). The species-specific antigenic fraction developed against SME alone is shown in DID, IE (Plate 6) as well as in CIE. The absorbed RAS (N) serum could detect native mutton to a minimum level of 10% when adulterated. Rabbit anti-goat native (RAG (N) testicular serum also showed three antigenic fractions with native GME amongst which one was species-specific (Plate 7). However, with cooked meat extract RAG (N) did not give any reaction either with homologous or heterologous cooked meat extract, thus indicating, antibody incompetence to react with heated antigen, as the antibody raised was, against native extract only. All the antisera raised in rabbits against native testicular antigens showed cross reactions, showing three to four antigenic fractions in each of the species, of which one was found to be species-specific. The reactions with homologous and heterologous muscle extract also indicated the serological relationship amongst all the four species (Pandey and Pathak 1975; Sherikar et al 1979).

As it was observed that there were common antigenic fractions in cattle, buffalo, sheep and goat, it was considered worthwhile to raise antisera in phylogenetically related hosts. Recent studies of Nath (1986), Bansal and Mandokhot (1988) and Reddy et al (1990) indicated the usefulness of such cross-immunization technique to develop species-specific antisera, which proved to be promising. In the present study, the antisera raised by cross immunization like buffalo anti-cattle, native (BAC (N), cattle anti-buffalo, native (CAB (N), goat anti-sheep, native (GAS (N) and sheep anti-goat, native (SAG (N) were proved to be monospecific even without absorption by giving a single line of precipitation in DID, IE and CIE (Plate 8) with homologous fresh muscle extracts only and similarly, these antisera could also detect cooked meats, when adulterated. BAC (N) serum detected at 20% level both in the case of fresh and cooked beaf, CAB (N) serum detected fresh (1%) and cooked (20%) cara beef, while GAS (N) serum detected fresh and cooked mutton up to 1% and 20% levels, respectively, and SAG (N), serum detected fresh (1%) and cooked (20%) chevon. It was observed that CAB (N), GAS (N) and SAG (N) sera were sensitive enough to detect adulteration at 1% level, and the same sera detected cooked meats only to a level of 20%. which indicated that the sera were raised against fresh testicular antigens and hence the result. However, a similar observation could not be recorded in BAC (N), as the buffalo being immunologically less evolved possibly. All these sera were lyophilised in 1 ml vials and when tested after two years, were found to be still reactive. The antisera raised in rabbits, which gave cross reactions indicated our assumption right, to develop antibody by cross immunisation in phylogenic species to avoid cross reactions.

All the antisera developed against heated (BE) antigens lost their reactivity within 10 days of storage at -20°C, possibly because of frequent thawing and freezing. This perhaps, could have been avoided, had the sera been inactivated at 56°C for 30min. Hence, only the ammonium sulphate precipitated antibody of different sera were found to be useful. All the sera developed against heated antigens in rabbits, showed cross reactions, which were successfully eliminated using glutaraldehyde. The absorbed RAC (BE) detected cooked beef to a minimum level of 5% and fresh beef to 10% when adulterated. Similarly, RAB (BE), RAS (BE) and RAG(BE) sera detected up to 10% and 20% levels of cooked and fresh cara beef, mutton and chevon, respectively in DID, IE and in CIE.

In rocket immunoelectrophoresis (RIE) (Plate 9) the shortest peak was observed with CME (N) followed by GME (N) and the longest for SME (N) and followed by BME (N). The zone of precipitation developed with BME (N) and GME (N) indicated four separate zones within the respective peak and in CME (N) and SME (N), two zones of precipitation within the peaks, when the experiment was conducted with RAB (N) serum. When RAC (N) serum was reacted in RIE (Plate 10), it was observed that there were five peaks in CME (N) and BME (N) and four peaks in SME (N) and GME (N), though not very distinct in the photograph. In RIE (Plate 11) RAS (N) serum showed the longest migrating peak formed zone or precipitation against BME (N), followed by CME (N) SME (N) and GME (N). Four peaks were seen in CME (N) and BME (N), whereas in the case of SME (N) and GME (N), the movement of the antigen was abortive and hence, only single peaks could be seen. RAG (N) serum developed different sized peaks of precipitation and three antigenic fractions with GME (N) and two for other muscle extract antigen. It was not possible to differentiate the meats of different species of origin by RIE even by the number of peaks within the peak itself, as they were not uniform in each experiment and hence the identification by RIE was inconclusive.

The concentric precipitation rings obtained with fresh buffalo meat against (CAB (N) serum were measured and the diameters were 11, 7, 5 and 4 millimeters, respectively for 20, 10, 5 and 2% levels of cara beef (Plate 12). By this method, the actual quantity of meat adulterated could be easily estimated by single radial immumodiffiusion (Fig. 2).

Plate 1. DID : Central wells A: RAB (N) B: RAC (N) C: RAG (N) D: RAS (N) sera. Peripheral wells 1: SME (N) 2: GME (N) 3: BME (N) and 4: CME (N) antigens

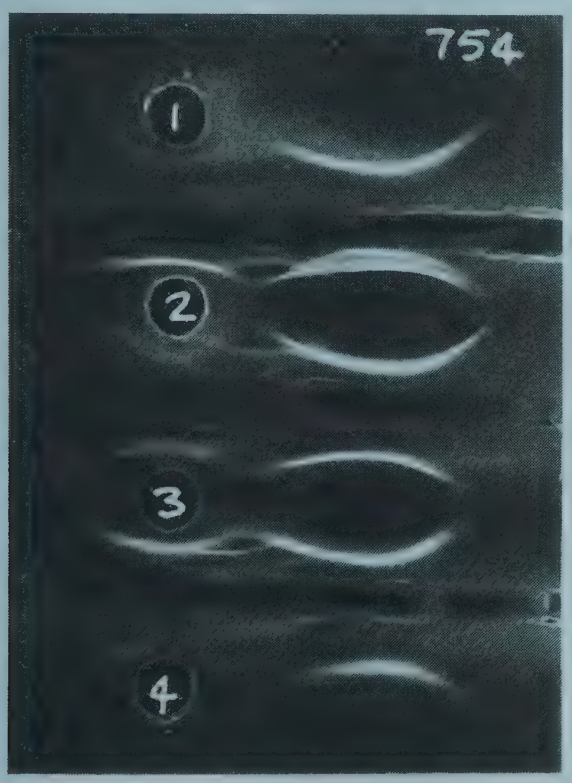

Plate 2. IE: All troughs RAC (N) serum. Wells 1: SME (N) 2: CME (N) 3: BME (N) and 4: GME (N) antigens

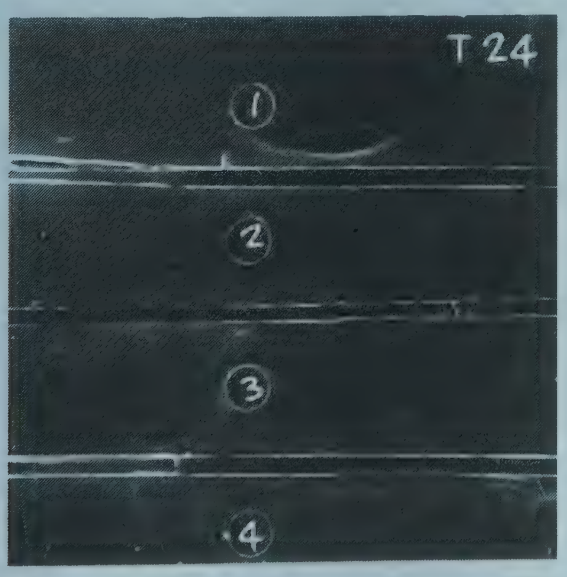

Plate 3. IE: All troughs RAC (N) serum. Wells 1: SME (N) 2: CME (N) 3: BME (N) and 4: GME (N) antigens

Plate 4. CIE: Right row wells RAC (N) serum. Left rows 1:CME (N) 2: BME (N) 3: SME (N) and 4: GME (N) antigens

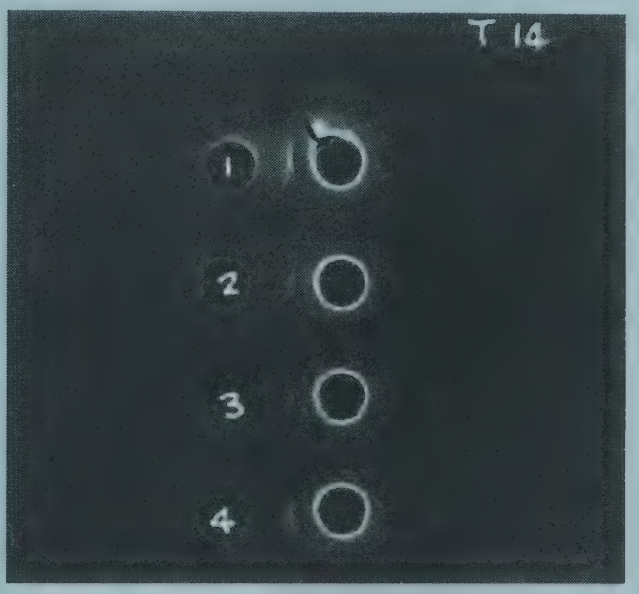

Plate 5. CIE : Right row first two wells from top to bottom RAB (N) serum and two wells from bottom to top RAB (H) serum. Left row wells 1: 20% BME (H) 2: 10% BME (N) 3: 20% BME (N) and 4: 10% BME (H) antigens

Plate 6. IE: All troughs RAS (N) serum. Wells 1: SME (N) 2: CME (N) 3: GME (N) and 4: BME (N) antigens

Plate 7. IE: All troughs RAG (N) serum. Wells 1: CME (N) 2: BME (N) 3: GME (N) and 4: SME (N) antigens

Plate 8. CIE: All rights row wells CAB (N) serum. Left row wells 1: BME (N) 2: CME (N) 3: SME (N) and 4: GME (N) 20% extracts

Plate 9. RIE: RAB (N) serum embedded in gel. Wells 1: CME (N) 2 BME (N) 3: SME (N) and 4: GME (N) antigens

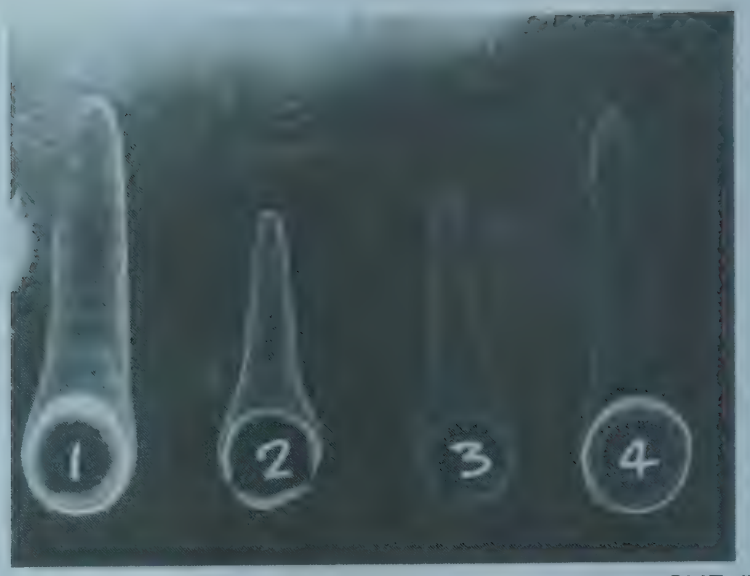

Plate 10. RIE: RAC (N) serum embedded in gel. Wells 1: CME (N) 2: BME (N) 3: SME (N) and 4: GME (N) antigens

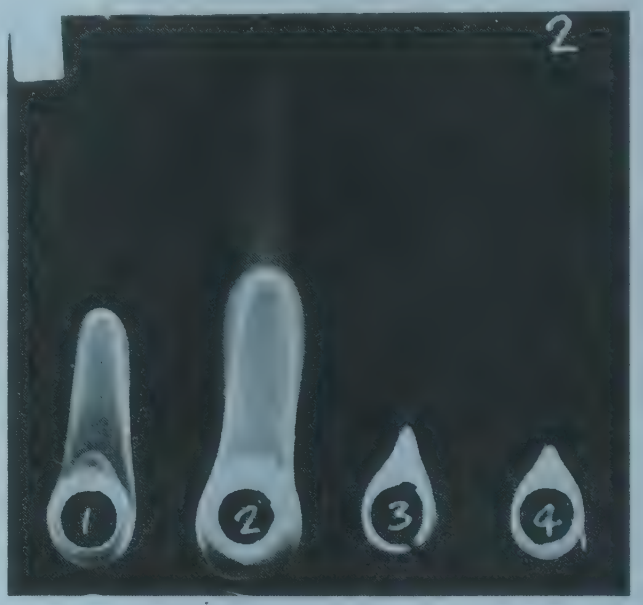

Plate 11. RIE: RAS (N) serum embedded in gel. Wells 1: CME (N) 2: BME (N) 3: SME (N) and 4: GME (N) antigens

Plate 12. SRID: CAB (N) serum embedded in gel. Wells 1:20, 2:10, 3:5, 4:2 percents of buffalo meat

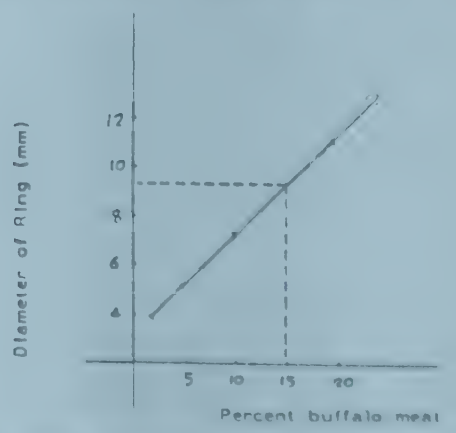

Fig. 2. Single radial immunodiffusion (SRID) quantification of adulteration

The sera obtained by cross-immunization, the BAC (BE) and CAB (BE) detected 10% and 20% minimum levels of cooked and native respectively of beef and cara beef when adulterated. However, GAS (BE) and SAG (BE) detected up to 20% only of either cooked or fresh mutton and chevon, respectively, either in DID, IE or CIE techniques. As the titres of the sera developed against heated (BE) testicular, antigens were found to be low and sera were needed to be concentrated in the present study. Efforts were also not made earlier to use antisera raised against heated (BE) antigens to detect native meat of different species origin and the present study showed the possibility of using antisera raised against heated (BE) antigen, for detection of native meats also. The problem of cross reactions and the difficulty in absorption to make the sera mono-specific was reported by several workers (Sherikar 1979; Swart and Wilks 1982). In the present study, it was overcome by simple immunoabsorption by cross linking the heterologous antigen with glutaraldehyde. The antisera raised against native testicular antigens in closely related species, behaved by themselves monospecific without the necessity of undertaking the laborious process of absorption, which is major contribution of the present work.

The antisera were stable even up to 24 months when freeze-dried and stored at -20°C, that made the reactants practically useful in the field. Wherever possible, it is recommended that CIE should be used to detect adulteration in fresh and cooked meats using the sera raised and alternatively, DID can also be used where facility of CIE does not exist. IE and RIE though sensitive, need further study before using routinely.

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RESEARCH PAPER

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Sensory and Yield Response Surface Analysis of Supercritical CO_2 Extracted Aromatic Oil from Roasted Coffee

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The CO_2 supercritical extraction of coffee oil from roasted Brazilian *Coffea arabica* was performed at different operational conditions. A central compositie design was then applied a 2^2 full factorial approach for two independent variables: pressure (250 to 300 bar) and temperature (50 to 90°C). Five levels with four star points and two replicates at the centre point were used. A sensorial panel composed of properly trained coffee consumers tested the characteristic coffee aroma of the roasted coffee aromatic oil extracted. The sensory analysis showed that the high temperature did not affect the intensity of characteristic coffee aroma and indicated that CO_2 supercritical extraction produced an extracted oil rich in coffee aroma. The yields of the extracted roasted coffee aromatic oil were analysed by the response surface analysis, as a function of the two experimental parameters. These parameters significantly affected the extraction yield. The best yield was obtained with decreases in the temperature values and pressure values at proximity of the centre point (275 bar).

Keywords : Coffee aroma, Coffee oil, Carbondioxide supercritical extraction, Response surface analysis, Sensory analysis.

Extraction with supercritical carbondioxide is a viable alternative to conventional processes for separation of biological materials, as it provides a final product generally with higher quality. Supercritical carbondioxide is an inert solvent, non-toxic, low cost, and its low critical temperature (31°C) permits efficient extraction of thermo-sensitive materials. Carbon dioxide also can be separated easily from the extracted products by simple evaporation.

The advantages and potentialities of liquid or supercritical carbondioxide extraction of coffee aroma have long been recognised. Schultz and Randal (1970) used liquid carbondioxide to extract aromatic constituents from roasted coffee. The extraction was performed at 25°C and at a pressure of 918 psi in a single step system. In 1974, a patent was filed for the production of vegetable extracts, more particularly for the production of tea and coffee extracts. The patented method was based on the liquid carbondioxide extraction (from 46 to 66 bar and from 12 to 25°C) of aromatic compounds from vegetable extracts, extraction of the soluble solids with water and addition of the aromatic extracts to the extracted soluble solids (Pictet et al. 1974).

In 1982, another extraction process for coffee oil containing aromatic constituents using supercritical carbondioxide was patented. It used in a closed circuit with pressures above 80 atm and temperatures above 31.3°C. The extraction yielded 10% of oil and antioxidant substances in addition to the aromatic compounds. Better yields (10-12% of coffee by weight) were obtained, when pressures between 200-400 atm and temperatures above 35°C were employed. A yellow to dark yellow extract was obtained, depending on the process conditions and with a very intense coffee aroma, when compared to oils obtained by mechanical extraction. (Roselius et al., 1982).

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Another 1995 patent reported that a unique aromatic product could be obtained by carbondioxide supercritical extraction of coffee aroma performed with pressures between 260-300 bar and temperatures between 60-90°C, 280 bar and 75°C, representing the best conditions. Higher yields in the aroma precipitation after extraction were obtained when applying a pressure drop to below 40 bar and temperature drop to below 15°C (Nichtnennung 1995).

Based on these results (Nichtnennung 1995), the present work was planned to study two operational conditions, pressure (P) and temperature (T), using an experimental design aimed at optimising the supercritical carbondioxide extraction yield and the intensity of the characteristic aroma of roasted Brazilian coffe arabica.

The aromatic composition of coffee is extremely complex, including aldehydes, ketones, heterocyclic compounds, esters, alcohols, sulphur compounds etc (Clarke and Macrae, 1985). A knowledge of the potential contribution of each aromatic compound to the odours and the odour of different combinations of these compounds is essential to the standardisation of coffee drink flavour (Semmelroch 1996). Considering this important factor in coffee quality, the roasted coffee aromatic oil obtained under different operational conditions of supercritical carbondioxide extraction was also subjected to sensory analysis for observation of the intensity of the characteristic coffee aroma. The oil extraction yields were analysed by response surface analysis, using an experimental factorial design as the tool. Factorial designs were introduced by G.E.P. Box in 50's. More recently, the application of factorial designs has grown exponentially because of the computer science revolution in statistical analysis (Barros et al. 1996).

Materials and Methods

Supercritical extraction of roasted coffee aromatic oil: Fig 1 illustrates the system used to extract aromatic oil from the

roasted coffee. Contact between the ground roasted coffee and the supercritical carbondioxide is promoted under established T and P conditions. The carbondioxide supplied from a gas cylinder (1) was liquefied through a cooling unit and compressed by a positive-displacement liquid pump (4) (Eldex, model AA-100-S) into a pre-heating extractor (9) immersed in a thermal water bath. The pressure and flow rate were controlled by micrometering valve (11) and the temperature was controlled by immersing the system in a thermal bath.

Extraction was carried out during 10 h with an average flow rate of carbondioxide expanded to atmospheric pressure of 2.071/min.

The operational conditions were defined according to the 2^2 experimental design with two independent variables, pressure (from 250 to 300 bar) and temperature (from 50 to 90°C). Five levels were considered with four star points and two replicates at the central point. Two dependent variables were obtained as responses, yield of extraction and intensity of characteristic coffee aroma. These variables are presented using regression and surface response analysis (Box et al. 1985; Barros et al. 1996). The experimental factorial design, which requires the use of coded variables, was used as a tool in the analysis of the influence of the operational variables (P and T) on the extraction yield and on the intensity of the characteristic coffee aroma.

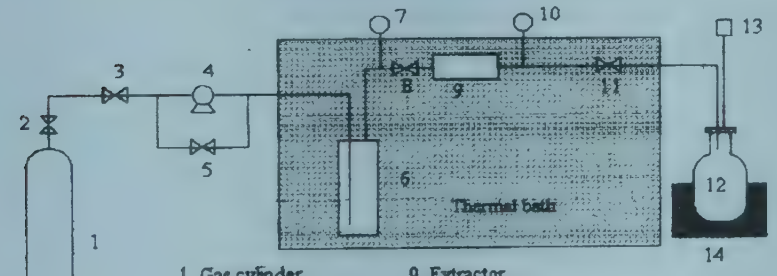

Fig. 1. Supercritical CO_2 aromatic oil extraction system for roasted coffee

Name : _____ Date : ___/___/___ Section : _____

Please evaluate the intensity of the characteristic coffee aroma in each of the coded samples, from the left to the right, and express your impressions using the scale below.

Sample

_____ |_____|
Weak Strong

_____ |_____|
Weak Strong

_____ |_____|
Weak Strong

Comments : _____

Fig. 2. Evolution card used in the selection of candidates for the tasting panel

Sensory analysis : A sensory team, of coffee consumers previously selected and trained, performed the analysis of the intensity of the characteristic coffee aroma for the roasted coffee aromatic oil obtained under different operational conditions. The coffee consumers were recruited and selected based on their individual abilities to discriminate differences in intensity of the characteristic coffee aroma.

In the first training period, each member of the team, the taster, was familiarised with the characteristic coffee aroma. To understand the meaning of the attributes, they would be measuring, they received three samples of ground roasted coffee, diluted in boiling distilled water, in proportions of 1.0, 5.0 and 15.0 g of coffee power to 50 ml of water, thus forming the individual smell perception relative to the intensity of the characteristic coffee aroma (weak, medium and strong respectively).

In a second session, the tasters were requested to evaluate the intensity of the characteristic coffee aroma in three samples. These samples were prepared by diluting the coffee in boiling distilled water in the proportions of: 2.0, 5.0 and 10.0 g of coffee to 50 ml of water. The taster was required to classify the samples using a card with a non-structured scale (9 cm) ranging from weak to strong terms (Fig. 2). The samples coded with three digit numbers were offered in tulip chalices wrapped in aluminium paper and covered with a watch glass, and evaluated in individual cabins with a red light. Each taster analysed each sample for three times.

With the results of each taster (variation sources: sample and repetition), an analysis of variance (ANOVA) was performed. The significance level (p) was calculated for each taster for the sample (F_{sample}) and the repetition ($F_{repetition}$). The tasters, who presented ($P_{sample} \leq 0.05$, $P_{repetion} \geq 0.30$) and concomparable results with the other panel members (based on a comparison of means), were selected to form the sensorial panel (ASTM 1981). Tables 1 and 2 show the statistical results obtained by the tasters in the training stage and selection of the sensory team.

Although, the training period was short, the results indicated that several tasters (1, 2, 6, 8, 10, 12, 15, 16, 17) had an excellent capacity to discriminate intensity differences in characteristic coffee aroma ($p \leq 0.05$), and good perception in their judgements ($p \geq 0.30$). These panel members performed the sensory evaluation of the roasted coffee aromatic oil obtained under the different operational conditions, using the same card used in the training and selection (Fig. 2).

Choice of solvent for the dilution of the roasted coffee aromatic oil : It was necessary, for the first study, to select a solvent that would better extract the compounds present in the roasted coffee aromatic oil. Three solvents used were acetone, ethyl acetate and dichloromethane, and 5 ml of each solvent was added to 1 ml of roasted coffee aromatic oil. The resulting solution (2 µl) was then injected onto the gas chromatograph (HP 5890 series III) with a HI Ultra 2 (25 m × 0.2 mm × 0.33 µm) capillary column. The chromatographic conditions for analysis were: initial temperature of 60°C with a gradual increase of 10°C per min. up to 190°C, and then a gradual increase was

TABLE 1. SIGNIFICANCE LEVEL (P) OF F_{sample}[1]/$F_{repetition}$[2] FOR EACH TASTER

	Tasters																
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Source	0.0064	0.0299	0.0595	0.1561	0.0004	0.0134	0.1200	0.0217	0.2351	0.0002	0.1500	0.0037	0.2285	0.0688	0.0159	0.0159	0.000*
Repetition	0.4883	0.9097	0.9875	0.7775	0.1333	0.8825	0.5717	0.6488	0.8556	0.4338	0.4513	0.5095	0.7015	0.6000	0.3551	0.2576	0.4756

[1]p of F_{sample} ≤ 0.05 shows tasters with adequate discriminative power

[2]p de $F_{repetition}$ ≥ 0.30 shows tasters with adequate repeatability

TABLE 2. SIGNIFICANCE LEVEL (P) OF F_{sample}[1]/$F_{repetition}$[2] FOR EACH TASTER

	ST#	Tasters*									ST#	Tasters*							
Sample*		1	2	3	4	5	6	7	8	9		10	11	12	13	14	15	16	17
1	1.87[a]	2.33	2.20	1.87	2.50	280	2.17	0.50	1.77	0.77	1.87[a]	0.90	1.77	0.57	3.20	2.67	2.73	1.67	0.40
2	4.09[b]	4.57	3.13	2.97	3.83	4.93	6.13	5.47	2.40	2.97	4.09[b]	4.20	5.87	2.83	5.13	2.70	3.00	5.77	4.50
3	6.94[c]	6.50	6.93	8.40	6.90	6.70	6.90	6.87	7.67	3.03	6.94[c]	7.77	6.60	8.47	6.73	5.50	7.23	8.03	7.80

* Sample 1 = 2.0 g of coffee; sample 2= 5.0 g of coffee; sample 3 = 10.0 g coffee (diluted to 50 ml water)

averages with different letters in the same column are significantly different at the ≤ 0.05 level of significance

of 5°C per min. up to a final temperature of 300°C, remaining at this temperature for 5 min. The injector temperature was 200°C and that of the detector was 250°C.

Fig. 3 shows that, basically, the same set of products was extracted by the three solvents with almost the same relative concentrations, but for absolute concentration, ethyl acetate gave the best results. Additionally, when 10 µl of the three samples were applied to an adsorbent strip of paper for aroma

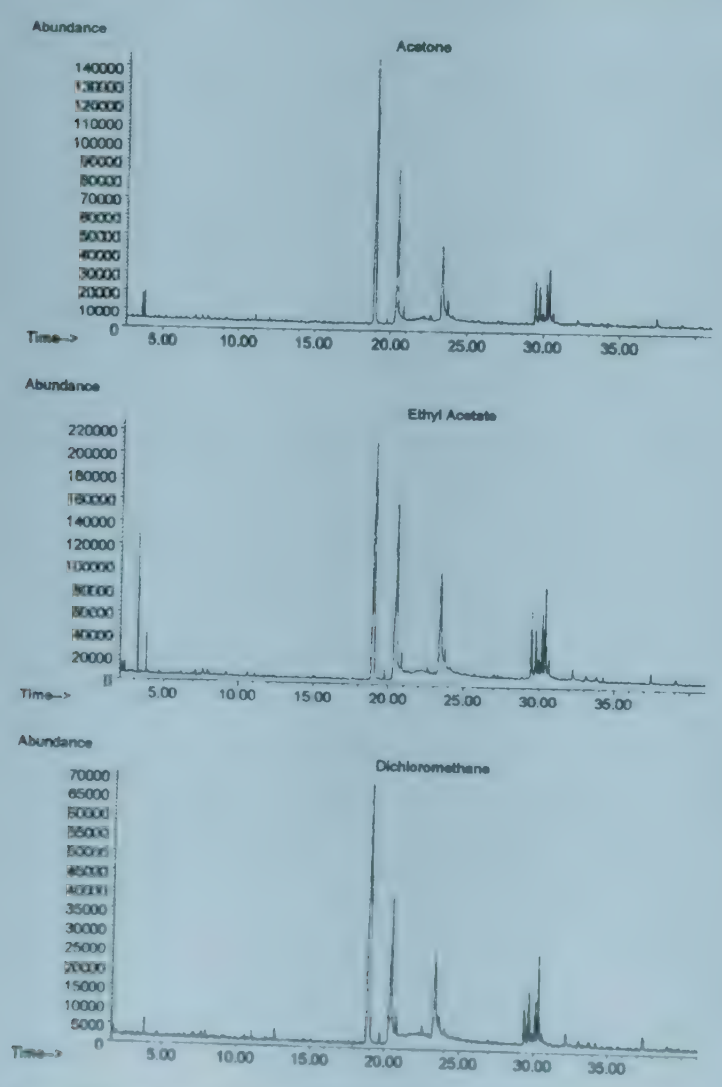

Fig. 3. GC/MS total ion chromatogram of roasted coffee aromatic oil diluted in acetone, ethyl acetate and dichloromethane

analyses, the roasted coffee aromatic oil diluted in ethyl acetate showed the greatest intensity of characteristic coffee aroma.

Sensory analysis of roasted coffee aromatic oil extracted by supercritical carbondioxide : Ten samples of roasted coffee aromatic oil obtained under different operational conditions (Table 3) and diluted in ethyl acetate (1:5), were applied to adsorbent strips of paper coded with three digit numbers for the evaluation by the sensory panel. In case, 10 µl of extract was transferred to an adsorbent strip, the taster was requested to taste this material after evaporation of the solvent. The time lapse between the application of the extract to the paper and evaluation by the taster was properly monitored, so as to be the same for all tasters in all repetitions. The samples were analysed in individual cabins with a red light. Each taster analysed each of the samples in three repetitions.

The results were analysed by an analysis of variance (ANOVA) and Tukey's test to determine any significance differences ($p \leq 0.05$) in the intensity of characteristic coffee aroma of the roasted coffee aromatic oils obtained under different operational conditions.

Results and Discussion

The results of each experiment based on the experimental factorial design are presented in Table 3.

Verification of a predictive model and analysis of the response surface for the extraction yield of roasted coffee aromatic oil by supercritical CO_2 : For the statistical treatment of the experimental data of extraction yield and to generate the response surface, a quadratic regression model was performed. Table 4 presents the coefficients of the predictive equation for the extraction yield of the roasted coffee aromatic oil as a function of the independent variables (P and T) studied, as well as its significance levels.

The regression coefficients that failed to present statistical significance ($p \leq 0.05$) were not considered in the model and incorporated in the error. Starting from this analysis, the statistical model was adjusted using coded values, which resulted in the following equation:

$$Y = 6.8963 - 0.763A - 0.4266B^2 \quad .....(1)$$

where, Y = the yield of the extraction (%).

The validity of the model (Equation [1]) was verified by the analysis of variance and presented in Table 5.

The value of the calculated F test compared to the tabulated value for the distribution F at 95% of confidence with the respective degrees of freedom showed that, for the yield of extraction in which $F_{regression/residue}$=15.35, the value was 3.24 times greater than the tabulated F. This fact in association with the value for the determination coefficient (R^2), indicated that the fitted quadratic model was statistically valid and had good predictive capacity.

Fig. 4 shows the behaviour of the experimental values as compared to the predicted values. It can be seen that the statistical model (Equation [1]), adjusted to the experimental values, can correctly predict the behaviour of the extraction yield with the variation in the independent variables (T and P) in the range studied.

With the statistical model adjusted for the experimental values of the extraction yield (Equation [1]) it was possible to generate the response surface that represented the influence of the operational variables on the yield (Fig 5). It can be seen that the yield increased with increase in pressure up to the proximity of the central point and that the effect of the temperature was more significant than that of the pressure. A decrease in the temperature considerably increased the extraction yield. Low temperature values associated with pressure values in the proximity of the central point were preferential to increase the yield.

Verification of a predictive model for the intensity of characteristic coffee aroma : Table 3 presents the results obtained by the selected trained sensory panel, for the intensity of characteristic coffee aroma for the different samples of roasted coffee aromatic oil extracted by supercritical carbondioxide.

Table 6 presents a quadratic regression model that was applied in the statistical treatment of the experimental data for the intensity of characteristic coffee aroma. The coefficients of the predictive equation of the experimental values for the

TABLE 3. EXPERIMENTAL DESIGN AND RESULTS FOR YIELD AND INTENSITY OF CHARACTERISTIC COFFEE AROMA.

Experimental number	Coded value A	B	T, °C	P, bar	Yield, %	Results Intensity of characteristic coffee aroma** (Tukey)*
1	-1	-1	50	250	6.95	4.85^{ab}
2	-1	-1	50	300	7.51	5.38^{ab}
3	.1	-1	90	250	6.00	3.98^{b}
4	1	1	90	300	6.00	5.57^{a}
5	-α	0	42	275	7.75	5.33^{ab}
6	α	0	98	275	5.17	6.25^{a}
7	0	-α	70	240	5.52	6.05^{a}
8	0	α	70	310	6.26	5.40^{ab}
9	0	0	70	275	7.28	5.99^{a}
10	0	0	70	275	7.09	5.53^{a}

α = 1.4142 at the star points

* averages with letters in common do not differ significanly at the level of significance of ≤ 0.05

** intensity scale where 1 = weak and 9 =strong

TABLE 4. REGRESSION COEFFICIENTS ESTIMATED FROM THE EXTRACTION YIELDS

Factor	Regression coefficient	t	p
Mean	7.18888*	26.35221*	0.000012*
Temperature (L)	-0.76149*	-5.59221*	0.005017*
Temperature (Q)	-0.25349	-1.41126	0.231005
Pressure (L)	0.20299	1.49075	0.210285
Pressure (Q)	-0.53785*	-2.9432*	0.040167*
Temperature × Pressure	-1.40000	-0.72573	0.508188

*coefficient p≤ 0.05 was selected to compose the quadratic model, p= significance level of t values

TABLE 5. ANALYSIS OF VARIANCE OF THE REGRESSION

Source of variation	Sum of squares	Degrees of freedom	Mean square	'F' test
Regression	5.7008	2	2.8504	15.35
Residue	1.2996	7	01857	
Unfit	1.2815	6	0.2136	11.80
Error	0.0181	1	0.0181	
Total	7.0004	9		
Determination coefficient R^2	0.8144			

$F_{0\ 95,2,7}$ = 4.74; $F_{0.95,6,1}$ =234.00

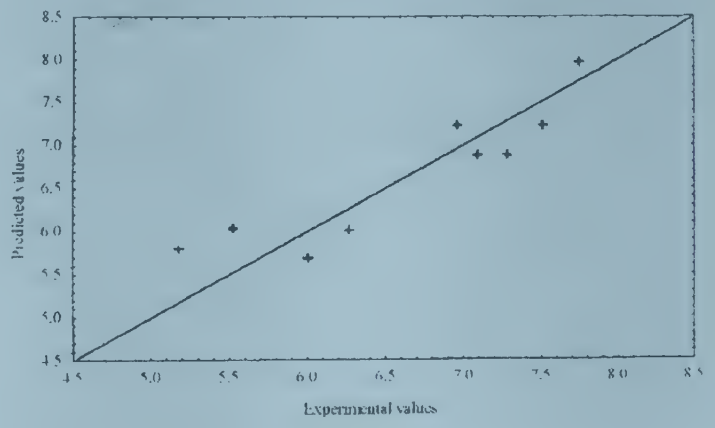

Fig. 4. Diagram of the experimental values against the values predicted by the quadratic model for the extraction yield of roasted coffee aromatic oil by supercritical CO_2

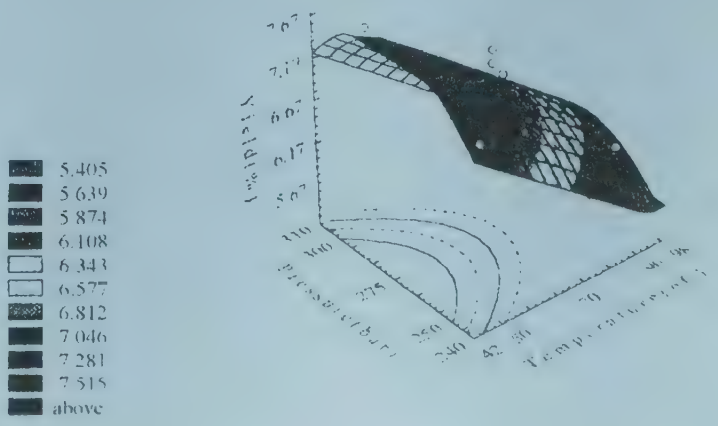

Fig. 5. Effect on T and P on the extraction yield

TABLE 4. REGRESSION COEFFICIENTS ESTIMATED FROM THE EXTRACTION YIELDS

Factor	Regression coefficient	t	p
Mean	5.75556*	9.281110*	0.000750*
Temperature (L)	0.079355	0.256359	0.810318
Temperature (Q)	-0.183256	-0.448797	0.676818
Pressure (L)	0.150769	0.487065	0.651698
Pressure (Q)	-0.218803	-0.535853	0.620469
Temperature × Pressure	0.265000	0.604299	0.578246

*coefficient p≤ 0.05 was selected to compose the quadratic model, p= significance level of t values

intensity of characteristic coffee aroma showed the function of the independent variables (P and T), as well as its level of significance.

It can be seen from Table 6 that none of the regression coefficients relative to the independent variables (P and T) was statistically significant ($p \leq 0.05$). Hence, in the range of the independent variables studied, although a significant influence had been observed in the yield of the supercritical extraction of roasted coffee aromatic oil, none of them significantly ($p \leq 0.05$) affected the intensity of the characteristic coffee aroma.

Considering the hypothesis that high temperatures could affect the intensity of characteristic coffee aroma in the roasted coffee aromatic oil extracted, high temperatures were used in this study (90 and 98°C). But, this effect was not observed in the sensory analysis.

The value for the intensity of characteristic coffee aroma (Table 3) was almost 6.0, a value considered as moderate in the evaluation score (Fig. 2). This indicated that in carbondioxide supercritical extraction, the roasted coffee aromatic oil was rich in characteristic coffee aroma even when high temperatures were used in different extraction processes, for example: roasted coffee oil obtained by the mechanical pressing of the beans.

The mechanical pressing of beans is a commonly employed process in Brazil by soluble coffee industries. The roasted coffee oil extracted by this process presents a non-characteristic coffee smell (burned) due to pyrolysis when high temperatures are used (Roselius et al., 1982).

When the ANOVA test followed by the Tukey test was applied to the experimental values of the sensory analysis, it was observed that sample 3, corresponding to the extraction process with a temperature of 90°C and pressure of 250 bar, differed significantly from samples 4, 6, 7, 9 and 10 with a lower intensity of characteristic coffee aroma. However, this fact cannot be explained by an influence of the independent variables (P and T), since samples obtained with pressure and/or temperature values greater than 250 bar and 90°C were shown to have greater intensities of characteristic coffee aroma.

This study was carried out a based on the patent filed by Nichtnennung (1995). The best yield for supercritical carbondioxide extraction of coffee aromatic oil was obtained when the values of the operational variables were: 280 bar and 75°C. The response surface analysis (Fig. 5) showed that the yield of the supercritical extraction of roasted coffee aromatic oil in the proximity of these values was relatively high. However, enlarging the range analysed (through a factorial design) it was possible to observe that a decrease in the temperature considerably increased the yield of the supercritical extraction. This process is economically convenient, since lower temperatures degrade the aromatic compounds less and the energy consumption is reduced.

The sensory analysis showed that higher temperatures did not significantly affect the intensity of the characteristic coffee aroma. If, in the range of temperatures and pressures studied, high values for temperature were shown to improve yield in the supercritical extraction, certainly there would be no serious problems with decomposition of the aromatic fraction of the roasted coffee oil. However, in this case, lower temperatures gave the best yields.

The data genarated from this study would be therefore, useful for future studies on the solubility of roasted coffee aromatic oil in supercritical carbondioxide.

Acknowledgement

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RESEARCH PAPER

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Dissipation of Alphamethrin Residues in/on Brinjal and Tomato During Storage and Processing Conditions

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Brinjals and tomatoes were sprayed with recommended dose (0.005%) of alphamethrin upto drenching level and stored at ambient (40°C) and refrigerated (5°C) conditions. Effects of storage, washing and boiling on reduction of residues were studied by gas chromatography, equipped with capillary column. Dissipation of alphamethrin was observed faster at room temperature as compared to cold conditions in both the vegetables. Washing and cooking were not very effective in lowering residues in brinjals. However, reduction of residues was more due to cooking than simple washing (25 to 33%). In tomato, both the processes reduced the residues almost to the same extent (11 to 30%).

Keywords : Gas liquid chromatography, Residues, Capillary column, Kitchen processes, Dissipation, Tomato, Brinjal.

Vegetables are served as side dishes and form essential components of human diet. Among vegetables, brinjal and tomato are very common and available almost throughout the year. These vegetables are prone to insect attack and thus get spoiled easily (Patel et al. 1994; Dikshit et al. 1980). To combat insect-pests and disease of these crops and to achieve higher production, many pesticides are used. In this respect, synthetic pyrethroids are of great importance because of their minimum deleterious effects namely, quick degradation in soil, virtually zero mobility in soil and rapid metabolism and excretion by animals. Among this group, a relatively new synthetic pyrethroid i.e., alphamethrin [A racemate of (S)-α-cyano-3-phenoxybenzyl (1R)-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropane-carboxylate and (R)-α-cyano-3-phenoxy-benzyl (1S)-cis-3-(2,2-dichlorovinyl)-2,2-dimethyl-cyclopropanecarboxylate] is gaining importance as insecticides in agriculture.

The present study was planned to generate data on the persistence of alphamethrin residues in brinjal and tomato stored at room temperature as well as under refrigerator conditions and the effect of washing and boiling/cooking on lowering of residues.

Materials and Methods

Marketable sizes tomato and brinjal were procured from the control field and sprayed with the recommended dose i.e., 0.005% alphamethrin up to drenching level. After 1 h, the treated samples, each of brinjal and tomato, were divided into two lots. One lot was kept at room temperature (40-42°C) and the other in refrigerator (5°C) to study their persistence and effect of different heat and wash processing.

Samples of both the vegetables were processed on 0 (1h), 1 and 3 days after treatment. On each time interval, 2 to 3 brinjals, and 4 to 5 treated tomatoes were chopped into small pieces. After thorough mixing and quartering, 20 g representative sample of each vegetable was extracted with 100 ml acetone after shaking for 1 h on a mechanical shaker. The organic phase was concentrated to 10 ml after filtering through anhydrous sodium sulphate. The extract was concentrated on a rotary vacuum evaporator at 40°C and cleaned by passing through a glass column packed with 5 g anhydrous sodium sulphate and activated silica gel (60-120 mesh): charcoal mixture (5:1, w/w). After pre-wetting the column with 80 ml hexane: acetone (4:1, v/v), the residue was eluted with 125 ml hexane: acetone mixture (4:1, v/v) and the eluate was concentrated on flash evaporator to 2 ml for GLC analysis (Malik et al. 1999).

To study the effect of washing, whole fruits were washed under running tap water for 30 sec. While in case of boiling/cooking, representative sample was taken in conical flask with 10 ml water in case of tomato and 15 ml in case of brinjal and boiled at 80 to 85°C for 10 to 20 min. The processed samples were extracted and cleaned in a similar way employed for fresh samples.

The alphamethrin residue was determined by gas liquid chromatograph (Hewlett Packard-5890A) equipped with (ECD, ^{63}Ni) Split injection system and fused silica capillary column (SPB-5), 30 m × 0.32 µm ID 0.25mm film thickness of poly (5% diphenyl/95% dimethyl) silioxane as stationary phase, GLC operating conditions were as follows: carrier gas flow (nitrogen) through column 2 ml min^{-1}; auxillary gas flow, 60 ml min^{-1}, injector temperature, 230°C; dectector temperature 290°C, oven temperature programme was 180°C (0 min) $\xrightarrow{20°C\ min^{-1}}$ 250°C(0 min) $\xrightarrow{5°C\ min^{-1}}$ 280°C (10 min); (Malik et al. 1999).

Prior to analysis of extracts for estimation of residues, recovery experiments were conducted on brinjals as well as on tomatoes. At fortification levels of 0.25 and 0.50µgg^{-1}, the respective recoveries recorded were 89.3 ± 2.65 and 85.00 ± 3.77% in brinjals and 95.60 ± 5.91 and 98.10 ± 9.36% in tomatoes.

Results and Discussion

Persistence of alphamethrin in/on brinjals and tomatoes: Under laboratory conditions (Table 1), the initial alphamethrin deposit of 0.322 µgg^{-1} came down to 0.267 and 0.172 µgg^{-1} in 1 and 3 days, showing 17 and 47 % dissipation, respectively (Fig. 1). The rate of dissipation was slow when compared with field conditions because under field conditions, the physical,

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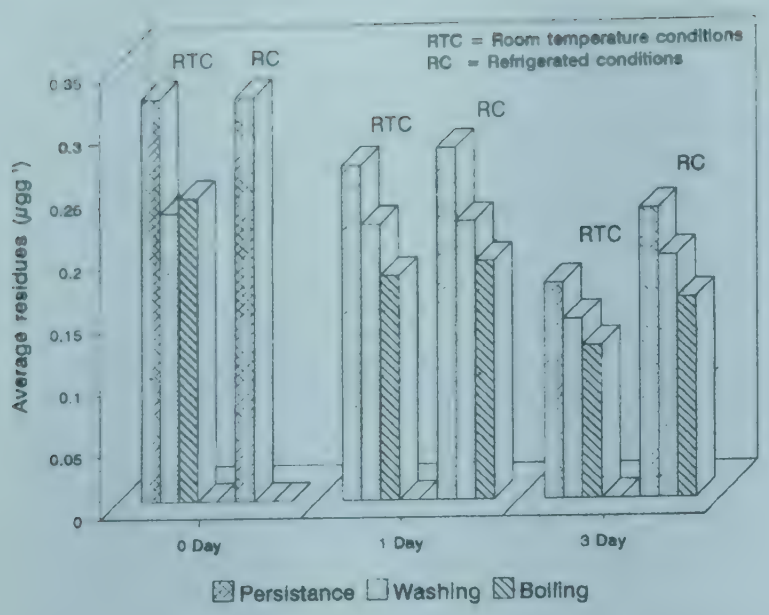

Fig. 1. Persistance of alphamethrin residue on brinjal and effect of washing and boiling on its reduction

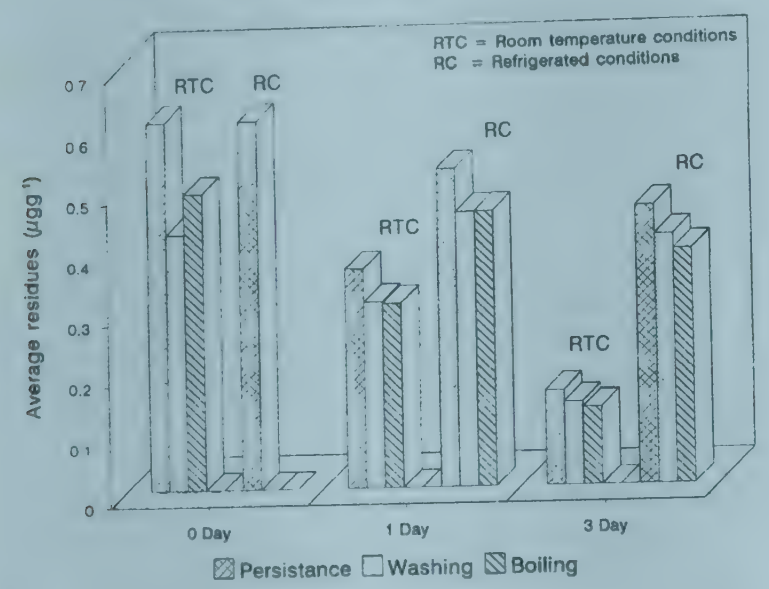

Fig. 2. Persistance of alphamethrin residue on tomato and effect of washing and boiling on its reduction

chemical and enzymatic activities were more intense. The similar initial deposits of synthetic pyrethroids on brinjals have been reported by Natekar et al (1988). The degradation process was quite slow, indicating the prolonged persistent nature of this insecticide. Under refrigerated conditions, dissipation was 13% after 1 day and 29 % after 3 days. The dissipation was almost 50% under cold conditions, which might be considered logical because low temperature decreased the enzymatic as well as chemical degradation of the insecticides. Gupta and Bhatnagar (1992) also reported that rate of dissipation of monocrotophos in tomatoes was much higher under field conditions than in deep-freeze storage conditions.

In tomatoes, stored under laboratory conditions, the initial deposit of 0.606 μgg^{-1} observed on 0 day dissipated by 41 % in 1 day, indicating 0.360 μgg^{-1} deposition, which further declined to 0.155 μgg^{-1}, indicating 74% dissipation after 3 days. It can be seen from the data (Table 1) that dissipation of alphamethrin was higher in case of tomatoes than brinjals, which could possibly be attributed to less penetration of the toxicant into the tomatoes (Fig.2). Sundarrajan (1983) reported that 90% permethrin dissipated from tomato fruits within a period of one week under field conditions. Under refrigerated conditions the initial deposit of 0.606 μgg^{-1} dissipated by 14%. after day 1 and 25% in 3 days, leaving 0.456 μgg^{-1} alphamethrin residues. Dissipation was less under cold storage conditions Similar observations have been made by Gupta and Bhatnagar (1992).

Effect of washing and boiling/cooking : Washing of brinjals stored at room temperature, reduced the residues to the extent of 29, 18 and 17% (Table 2) on 0, 1 and 3 days of treatment, respectively. For the same treatment, under refrigerated condition, the alphamethrin residues reduced from 0.280 to 0.222 μgg^{-1} after one day and from 0.230 to 0.193 μgg^{-1} after 3 days and washing, reduced 21 and 16% of the residues on the corresponding days. Under both the sets of conditions, washing was found to be more effective on 0 day, where the process could dislodge surface residues to the extent of 28 %. Although washing became less effective with duration of storage, it was almost identical in brinjals stored at room temperature and refrigerated conditions. In both conditions of storage, washing reduced the residues from 16-17 % in 1 and 3 day samples, indirectly that storage conditions did not effect adherence of the residues on the surface of the fruit or penetration into the fruit. The less effectiveness of washing could be attributed to penetration of chemial into pulp of fruit (Fig. 1). Reduction of fenvalerate and decamethrin from 29 to 51 % due to washing on brinjals has been reported by Raha et al (1993). They further observed that with the passage of time, the washing became less effective in reducing surface residues.

Washing of tomato samples stored under room temperature reduced the residues by about 31% in 0 day, 15% in 1 day and 12% in 3 day samples. Washing of samples stored under cold condition reduced the residues by 13 % in 1 days samples and 10% in 3 days. As evident from the data (Table 2), washing had the maximum effect on 0 day samples but its

TABLE 1. PERSISTENCE AND DISSIPATION OF ALPHAMETHRIN RESIDUES IN/ON BRINJALS AND TOMATOES

	Brinjal		Tomato	
DAT	Room temperature	Refrigerator	Room temperature	Refrigerator
0	0.322 ± 0.075	–	0.606 ± 0.052	–
1	0.267 ± 0.078 (17.08)	0.280 ± 0.090 (13.04)	0.360 ± 0.058 (40.59)	0.522 ± 0.048 (13.86)
3	0.172 ± 0.071 (46.58)	0.230 ± 0.029 (28.57)	0.155 ± 0.036 (74.42)	0.456 ± 0.068 (24.75)

DAT-Days after treatment

The above values are average of three replicates in μgg^{-1}

Parenthesis shows percent dissipation

TABLE 2. EFFECT OF WASHING ON THE REDUCTION OF ALPHAMETHRIN RESIDUES IN/ON BRINJALS AND TOMATOES

DAT	Brinjal				Tomato			
	Room temperature		Refrigerator		Room temperature		Refrigerator	
	Before washing	After washing	Before washing	After washing	Before washing	After washing	Before washing	After washing
0	0.322 ± 0.075	0.231 ± 0.028 (28.26)	-	-	0.606 ± 0.052	0.422 ± 0.026 (30.36)	-	-
1	0.267 ± 0.078	0.220 ± 0.109 (17.60)	0.280 ± 0.090	0.222 ± 0.069 (20.71)	0.360 ± 0.058	0.305 ± 0.009 (15.28)	0.522 ± 0.048	0.451 ± 0.065 (13.60)
3	0.172 ± 0.071	0.143 ± 0.025 (16.86)	0.230 ± 0.029	0.193 ± 0.024 (16.08)	0.155 ± 0.036	0.136 ± 0.038 (12.26)	0.456 ± 0.068	0.408 ± 0.074 (10.53)

DAT-Days after treatment

The above values are average of three replicates in μgg^{-1}

Parenthesis shows percent dissipation

TABLE 3. EFFECT OF BOILING ON THE REDUCTION OF ALPHAMETHRIN RESIDUES IN/ON BRINJALS AND TOMATOES

DAT	Brinjal				Tomato			
	Room temperature		Refrigerator		Room temperature		Refrigerator	
	Before boiling	After boiling	Before boiling	After boiling	Before boiling	After boiling	Before boiling	After boiling
0	0.322 ± 0.075	0.243 ± 0.002 (24.53)	-	-	0.606 ± 0.052	0.488 ± 0.044 (19.47)	-	-
1	0.267 ± 0.078	0.179 ± 0.043 (17.60)	0.280 ± 0.090	0.190 ± 0.010 (32.14)	0.360 ± 0.058	0.302 ± 0.088 (15.11)	0.522 ± 0.048	0.452 ± 0.112 (13.41)
3	0.172 ± 0.071	0.122 ± 0.047 (29.06)	0.230 ± 0.029	0.159 ± 0.018 (30.87)	0.155 ± 0.036	0.127 ± 0.092 (18.06)	0.456 ± 0.068	0.384 ± 0.069 (15.79)

DAT-Days after treatment

The above values are average of three replicates in μgg^{-1}

Parenthesis shows percent dissipation

effect got reduced with the passage of time being slightly more under room temperature conditions than the cold conditions (Fig. 2). Reduction of fenvalerate residues on tomatoes due to washing has been reported to be 62% by Jain et al. (1979) and 38% by Sances et al. (1992).

The initial deposit of 0.322 mgg^{-1} (Table 3) in/on brinjals declined to the level of 0.243, 0.179 and 0.122 mgg^{-1} after boiling/cooking on 0, 1 and 3 days, showing 25, 33 and 29% reduction on the corresponding days at room temperature conditions. Under refrigerated conditions, the residues got reduced by 32 and 31% after 1 and 3 days, respectively. Here also, the effect of boiling on reduction of alphamethrin residues was almost same under both storage conditions (Fig.1). Hotellier (1982) reported that deltamethrin residues reduced appreciably on cooking. Reduction of fenvelerate residues to the extent of 27.30 to 56.12% in/on brinjals was reported by Sharma and Kumar (1993). It clearly indicated that boiling could reduce residues substantially. Relatively, less effect of boiling in the present experiment could possibly be attributed to thermal stability of the alphamethrin.

As observed in brinjals, boiling was not very effective in reducing alphamethrin residues in tomatoes, probably because of thermal stability of alphamethrin. Reduction of residues of alphamethrin due to boiling of tomato was to the extent of 19% on 0 day, 16-18% in 1-3 day samples stored at room temperature and 13-16% in samples stored under cold conditions (Fig. 2). Contrary to the present findings, Jain et al. (1979) observed 68 to 70 reduction of fenvelorate residues due to cooking of tomatoes collected 1 h after the spray.

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RESEARCH PAPER

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Studies on Selected Functional Properties of Defatted Sunflower Meal and its High Protein Products

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Defatted sunflower meal (DSM) and high protein products low in polyphenols and phytate from it were prepared and their selected functional properties were studied. The polyphenols and phytate were found decreased by 98.6 to 62.1 and 31.1 to 77.3% in protein concentrates and 67.0 to 98.6 and 92.5 to 90.5% in protein isolates, respectively. The nitrogen solubility of DSM and high protein products in water were similar with a minimum in the pH range of 4 to 6. The water and oil absorption, bulk density, gelation, foaming capacity and emulsification properties were found increased significantly ($p<0.05$) in high protein products over DSM. Among the high protein products, the protein isolates showed better functional properties than the protein concentrates.

Keywords : Defatted sunflower meal, Low polyphenol concentrate, Isolate, Reduced phytate concentrate, Functional properties, Nitrogen solubility.

Sunflower (*Helianthus annuus* L.) is a potential source of low cost protein for future. It contains about 20% proteins and 40 to 45 % oil. In terms of world production, sunflower is the fourth major oilseed, the third most important source of edible oil and the fourth largest source of food and feed proteins (Lusas 1985). The sunflower meal after extraction of oil is presently used primarily for feeding the ruminants. However, because of its unique nutritional, sensory and functional properties, the meal can be used as a potential source of protein in processed human foods.

Unfortunately, the major problem in using sunflower meal in human foods is the presence of hulls and polyphenols (Sosulski and Bakal 1969) and phytate (Cheryan 1980). The hulls contribute to high crude fibre content of the meal and also imparts dark colour (Wan et al. 1979).

Attempts have been made earlier to remove these polyphenolic substances to obtain colourless sunflower protein products from sunflower meal. Sodini and Canella (1977), Saeed and Cheryan (1988) and Prasad (1988) described the use of acidic butanol reagent for removing phenolics from sunflower meal without causing detectable protein denaturation. Rahma and Rao (1981) used water, 0.002 N HCl, 70 % ethanol, 2% NaCl solution, acidic n-butanol and multiple solvents for removal of polyphenols and reported increase in protein content and markedly decrease in polyphenols content in meal treated by all solvents. They further reported that organic solvents caused a decrease in nitrogen solubility, whereas water, 0.002 N HCl, 2 % NaCl solution caused an increase. Sripad and Rao (1987) used 40% each of aqueous acetone, ethanol and methanol to remove polyphenols from sunflower meal and reported lower nitrogen solubility of the extracted meals than that of unextracted meal in water in the pH range of 2 to 8.

The phytate in sunflower meal must also be reduced because of its well documented effect on nutritional and functional properties of proteins (Cheryan 1980). There are number of reports on the effect of removal of polyphenol from the sunflower meal on its functional properties (Lin et al. 1974; Kilara et al. 1972; Rahma and Rao 1981). However, there are no reports on the effect of polyphenol and phytate removal from the sunflower meal and its high protein products on the functional properties. Attempts were made in this study to produce high protein products low in polyphenols and phytate. Further, some of the functional properties of these products were also studied since not much information is available on derived products.

Materials and Methods

Preparation of defatted sunflower meal : The sunflower seeds (var. 'MSFH-8') were dehulled and meal (DSM) was prepared.

Preparation of low-polyphenol protein concentrate (LPC): The method of Sodini and Canella (1977) was used for the preparation of sunflower protein concentrate that was substantially free from polyphenol. The DSM was suspended in acidified butanol in a ratio of 1:20 (w/v), pH was adjusted to 5.0 with 0.6 N HCl and extracted, centrifuged, filtered and the residue (LPC) was dried under nitrogen at room temperature.

Preparation of reduced-phytate protein concentrate (RPC): The defatted meal was suspended in tap water in the ratio of 1:5 (w/v) and the pH adjusted to 5.0 with 0.5 N HCl, centrifuged, filtered and residue (RPC) was freeze dried.

Preparation of reduced-phytate protein isolate (RPI): The defatted meal was extracted in water, pH adjusted to 10.0 with 0.5 N NaOH for 30 min at a flour to solvent ratio of 1:15 (w/v). The suspension was centrifuged and pH of supernatant was brought to 5.0 with 0.5 N HCl (the point of maximum solubility of sunflower protein). Subsequently, the precipitate was neutralized to pH 7.0 with 0.5 N NaOH and freeze-dried.

Preparation of low-polyphenol reduced-phytate protein isolate (LPRPI) : The LPC was extracted with water and its pH was adjusted to 10.0 with 0.5 N NaOH for 30 min at a meal to solvent ratio of 1:15 (w/v), centrifuged, filtered and the residue was extracted once again under the same conditions. The two clear supernatants were combined and pH adjusted

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to 5.0 with 0.05 N HCl. The precipitate was separated by centrifugation, washed with acidified water at pH 5.0, slurried in water, neutralized to pH 7.0 with NaOH and freeze-dried.

Determination of proximate principles : The samples of DSM and high protein products were analyzed for crude proteins, crude fat and ash contents by the procedures of AOAC (1980).

Determination of polyphenols and phytate : Polyphenols were determined by the method of Dorrell (1976) and Price et al (1978) with slight modifications as described earlier (Pawar et al. 2000) and the phytate by the method of Makower (1970).

Determination of functional properties : Some of the important functional properties of defatted sunflower meal and its high protein products such as protein solubility (Narayana and Rao 1982) with slight modifications described by Pawar et al (2000), water and oil absorption capacities (Beuchat 1977), least gelatin concentration (Coffmann and Garcia 1972) with sligh modification as described by Deshpande et al (1982), bulk density (Wang and Kinsella 1986), foam capacity and stability (Coffmann and Garcia 1977), emulsifying activity and its stability (Yasumatsu et al. 1972) with slight modifications described by Dev et al (1986) and emulsifying capacity and stability (Beuchat 1975) were determined. Emulsification capacity determinations were carried out at room temperature and values are expressed as g of oil emulsified by 1 g of protein from the sample. Subsequently, a volume of oil equivalent to 80% of the emulsifying capacity was added to 10 ml of sample dispersion and blended for 10 sec on a Virtis blender as per the procedure of Thompson et al (1982). The emulsion was transferred to a 50 ml capacity graduated cylinder and emulsion stability was recorded in terms of % aqueous phase separated over a period of 1-4 days.

Results and Discussion

The data in Table 1 on proximate composition show that polyphenols and phytate contents deceased to a considerable extent in the high protein products. However, there was still a large quantity of phytate in LPC. The recovery of protein was to the extent of 70-73 % in concentrates, whereas, 92-94 % in isolates. Fat and ash contents were found decreased in both these high protein products. The reduced phytate concentrate and isolate were of dark brown colour as compared to that of low polyphenol concentrate and isolate. This difference in the colour might be due to binding of oxidized products of polyphenols to proteins. The results of the present with investigation coincided with those of Saeed and Cheryan (1988).

Nitrogen solubility is considered to be a good index of functional properties and data presented in Fig. 1 show that all the samples exhibited minimum nitrogen solubility in the pH range of 4.0 to 6.0. The nitrogen solubility increased on either side of this pH range and this was due to more charged sites on proteins. Unlike Kilara et al (1972) on soybean meal, a sharp minimum solubility at isoelectric point was not observed in DSM. Instead, a broad range of minimum solubility between pH 4.0 to 6.0 was found. This has been observed in sunflower meal by Gheyasuddin et al. (1970) who reported maximum extractability of nitrogen at pH 10.0 being about 90% soluble compared to 80% for soybean.

Data further show that LPC exhibited a lower nitrogen solubility than DSM over pH range examined. This may be due to the fact that the methods used for removal of polyphenols or preparation of the high protein products may preferentially remove low molecular weight proteins from the meal, which

TABLE 1. PROXIMATE COMPOSITION OF DSM AND PROTEIN PRODUCTS LOW IN POLYPHENOLS AND PHYTATE (% DRY BASIS)[a]

Sample	Crude protein, N × 6.25	Crude fat	Ash	Polyphenols, mg/100g	Phytate, mg/100 g
DSM	62.1	1.0	8.0	3.52	4.01
LPC	70.0	0.9	8.2	0.05	3.21
	(12.7)	(11.7)	(2.5)	(98.6)	(31.1)
RPC	73.5	0.9	2.9	1.25	0.91
	(18.4)	(8.8)	(68.7)	(62.1)	(77.3)
RPI	92.2	0.2	1.8	1.07	0.30
	(48.4)	(76.4)	(77.5)	(67.0)	(92.5)
LPRPI	94.1	0.23	1.8	0.05	0.38
	(51.5)	(77.4)	(77.5)	(98.6)	(90.5)

[a] = each value is average of three observations

DSM-Defatted sunflower meal; LPC-Low-polyphenol protein concentrate; RPC-Raduced-phytate protein concentrate; RPI-Reduced-phytate protein isolate; LPRPI-Low-polyphenol reduced-phytate protein isolate. Figures in parenthesis indicate % increase or decrease

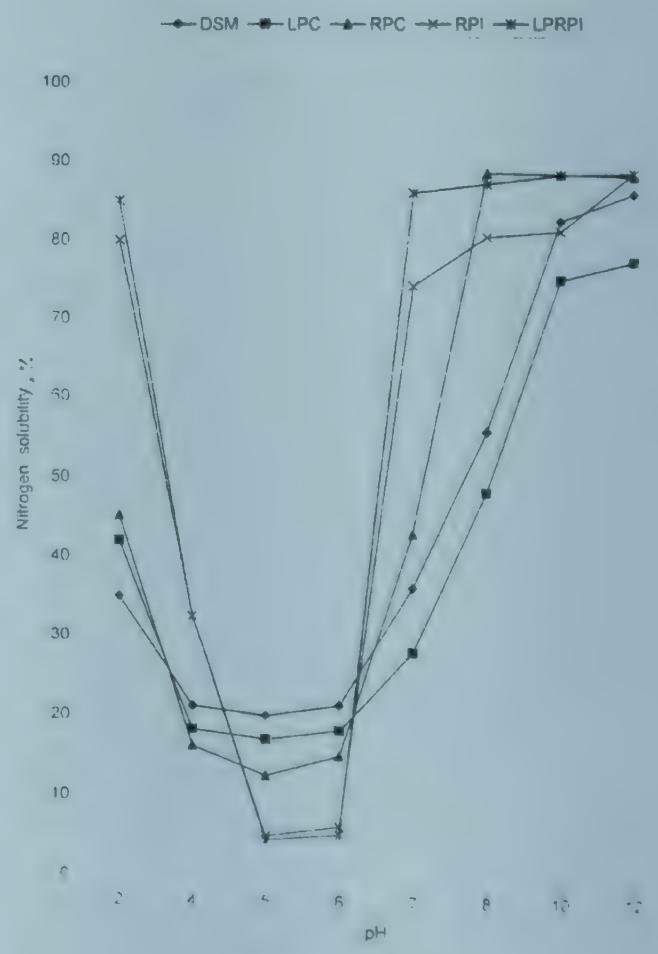

Fig. 1. Effect of pH on nitrogen solubility of defatted sunflower meal (DSM), low-polyphenol concentrate (LPC), reduced phytate concentrate (RPC), reduced phytate isolate (RPI) and low-polyphenol reduced phytate isolate (LPRPI)

may be more soluble. Moreover, an organic solvent as used for removal of polyphenols might have caused some denaturation of protein as reported earlier by Sodini and Canella (1977) and Rahma and Rao (1981). The broad range of minimum nitrogen solubility is due to interaction of polyphenols with the protein. Rahma and Rao (1981) reported an apparent increase in nitrogen solubility in sunflower meal after aqueous extraction of chlorogenic acid at pH 5.0. Rahma and Rao (1979) also reported a sharp minimum solubility point in extracted meal compared to a broad minimum solubility pH range for the unextracted meal. However, in the present study, this phenomenon could not be ascribed due to denaturing effect of organic solvent used on the soluble proteins.

The nitrogen solubility profile of LPRPI was found increased from 35.0% for DSM to 85.0 % for LPRPI. This product showed a sharp and minimum nitrogen solubility point near pH 5.0, probably because of loss of water soluble protein or non-protein nitrogen during preparation. This product also showed the highest nitrogen solubility at neutral pH than rest of the products.

The nitrogen solubilities of RPC and RPI were found higher than the DSM below the isoelectric point. This could be due to removal of phytate; because the reduced phytate products were found lower in phytate content (77.3 to 92.5%) than the DSM. It should be remembered that phytate is highly soluble at pH 5.0 and it is known to interact with proteins and cause significant shifts in the solubility profiles (Cheryan 1980). Rahma and Rao (1979) confirmed this, while characterising sunflower proteins. They observed shifting of isoelectric point from 5.0 to 6.0 and lowering of nitrogen solubility index of the extrac-ted meal with sodium chloride for polyphenol removal.

The reduced phytate products viz. RPC and RPI, however, showed increased nitrogen solubility at pH 2.0 and above pH 6.0. This could be, in the former case, due to significant amount of acid soluble whey protein, which was removed during the acid extraction step and in the later case, due possibly to removal of phytate, which can form ternary complexes with proteins that may be insoluble, or due to loss of alkaline-insoluble proteins during the extraction-centrifugation steps (Cheryan 1980).

The water and oil absorption increased significantly (Table 2) for both concentrates and much more significantly for isolates than the DSM, which may be due to reduction of polypehnols and phytate, which might have made more protein available for holding the water and oil. Lin et al (1974) and Rahma and Rao (1981) also observed an increase in water and fat absorption capacity of sunflower-treated meal. When the values were expressed on protein basis rather than on meal basis, the same trend was observed indicating thereby that the role of other water and fat binding constituents of sunflower meal did not play a significant role. It is well known that polyphenols and phytate interact with oilseed proteins and lower their availability. The least gelatin concentration was 10% in DSM, whereas it was 12 % in concentrates and 16% in isolates. The least gelation concentration of rapeseed meal and isolate reported by Dev and Mukharjee (1986) are more or less similar as in the present investigation. The high protein

TABLE 2. WATER AND OIL ABSORPTION CAPACITY, LEAST GELATION CONCENTRATION AND BULK DENSITY OF DSM AND PROTEIN PRODUCTS LOW IN POLYPHENOLS AND PHYTATE (%) DRY BASIS)[a]

Sample	Water absorption, g/g		Oil absorption g/g		Least gelatin concentration	Bulk density,
	Flour	Protein[b]	Flour	Protein[b]	w/v%	g/ml
DSM	2.00	3.22	1.80	2.89	10	0.657
LPC	2.57	3.60	2.30	3.28	12	0.428
RPC	2.75	3.74	2.50	3.40	12	0.243
RPI	4.20	4.55	4.50	4.88	16	0.190
LPRPI	4.54	4.82	4.80	5.10	16	0.201
S.E. ±		0.012		0.014	0.047	0.025
C.D. at 5%		0.040		0.048	0.148	0.830

[a]=each value is average of three observations; b-Expressed on crude protein basis; DSM-Defatted sunflower meal; LPC-Low-polyphenol protein concentrate; RPC-Raduced-phytate protein concentrate; RPI-Reduced-phytate protein isolate; LPRPI-Low-polyphenol reduced-phytate protein isolate

TABLE 3. FOAM CAPACITY OF 1% (W/V) AND ITS STABILITY OF DSM AND PROTEIN PRODUCTS LOW IN POLYPHENOLS AND PHYTATE (%) DRY BASIS)[a]

Sample	Volume after whipping, ml		% volume increase	Foam stability at room temperature after min				% volume decrease over 120 min	
	Total volume	Foam volume		30	60	90	120	Total volume	Foam volume
DSM	128	32	28	16	14	12	12	18.75	62.50
LPC	132	42	32	18	18	8	8	24.24	87.34
RPC	130	40	30	14	8	8	8	23.07	77.70
RPI	148	48	48	20	12	12	10	24.24	75.00
LPRPI	152	48	52	22	12	12	10	26.76	79.16
S.E. ±	0.656	0.140	0.390						
C.D. at 5%	2.060	0.480	1.280						

[a]=each value is a average of three observations; b-Expressed on crude protein basis; DSM-Defatted sunflower meal; LPC-Low-polyphenol protein concentrate; RPC-Raduced-phytate protein concentrate; RPI-Reduced-phytate protein isolate; LPRPI-Low-polyphenol reduced-phytate protein isolate

products should have exhibited, in fact, improved gelling property over DSM but did not do so because of various changes, which took place in other components during the preparation of these high protein products. Though they failed to exhibit the typical gelatin characteristics as to those of DSM but formed highly viscous, often immobile paste. The data on bulk density of all samples (Table 2) showed that DSM was more dense than the other sample. The difference in particle size may account for difference in the bulk density of flours and inverse relationship between oil absorption capacity of flour. The high protein products showed very less bulk density as compared to DSM, which may be due to the freeze-drying treatment of the sample.

The data on foaming property (Table 3) show that DSM produced least foam than the high protein products low in polyphenols and phytate, which were perhaps due to lower amount of protein and the presence of carbohydrate, chlorogenic

TABLE 4. EMULSIFICATION PROPERTIES OF DSM AND PROTEIN PRODUCTS LOW IN POLYPHENOLS AND PHYTATE (% DRY BASIS)[a]

Sample	Emulsifying activity		Emulsifying stability		Emulsifying capacity at pH 7.0, ml of oil/100g	% Aqueous phase at room temperature after time, days			
	Flour	Protein[b]	Flour	Protein[b]		1	2	3	4
DSM	51.5	82.9	58.3	93.9	25.5	70	80	84	84
LPC	53.3	76.1	62.2	87.4	33.6	50	80	80	80
RPC	57.7	78.5	63.8	86.8	36.8	20	50	70	70
RPI	62.0	67.3	70.3	76.2	43.3	00	00	00	00
LPRPI	66.6	70.8	78.5	83.4	45.5	00	00	00	00
S.E. ±	0.11		0.39		0.14				
C.D. at 5%	2.32		1.28		0.45				

[a]=each value is average of three observations; b-Expressed on crude protein basis; DSM-Defatted sunflower meal; LPC-Low-polyphenol protein concentrate; RPC-Raduced-phytate protein concentrate; RPI-Reduced-phytate protein isolate; LPRPI-Low-polyphenol reduced-phytate protein isolate

acid and phytate, which have depressing action on foaming properties. Kabirullah and Wills (1988) reported a positive correlation between protein solubility and foaming capacity in sunflower protein, which fairly coincided with the present observation. As seen in Table 3, foams from DSM and protein isolate were more stable than those obtained from protein concentrates. The per cent decrease over 120 min time under study was highest in concentrates and lowest in isolates. The stability of foam is independent to the amount and nature of protein present. The more the effect of protein, the more would be the foam stability; but opposite trend was observed and this could be probably because of some other components such as carbohydrates that might have played a role in stabilizing the foam in the DSM. The results are comparable with sunflower meal products Lin et al (1974). Rahma and Rao (1981) also reported increase in foaming capacity of sunflower meal extracted with acidic n-butanol.

The data presented in Table 4 show that the emulsifying activity was highest in LPRPI and lowest in DSM with intermediate values for rest of the samples. Additional heating (80°C for 30 min) revealed that 50 to 80 % of the original emulsified layer remained stable after processing the sample. The DSM showed less emulsion properties than other samples. The emulsion stability was found higher than emulsifying activity and variations in these values were primarily due to water loss during centrifugation.

The emulsifying capacity values as determined by titration method (Table 4) showed a somewhat different pattern compared with those of emulsifying activity and emulsion stability. The emulsifying capacity of the DSM was lowest and it increased significantly in high protein products. The emulsion prepared with RPI and LPRPI did not show separation of water up to 4 days. The DSM had a poor emulsion stability with 70% of water phase of its emulsion being separated within one day. The RPC showed less separation (20%) than LPC (50 %). The results in Table 4 further show that the reduced phytate products RPC, RPI and LPRPI exhibited superior emulsion properties than DSM as well as LPC.

It may be concluded that the high protein products low in polyphenols and phytate could be the very significant high protein sources of better functional properties to be used in the fortification of a number of processed foods.

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RESEARCH NOTE

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Effect of Temperature on Myofibril Fragmentation During Post-mortem Storage of Muscle

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Myofibrillar Fragmentation Index (MFI) was estimated in chicken, sheep, goat and cattle meat in order to determine the pattern of myofibrillar fragmentation on storage. The pH was measured after storing the muscles at 27±1°C for 24 h. The order of pH decline pattern was chicken > cattle > goat > sheep. The leg muscles of chicken and longissimus dorsi muscles of sheep, goat and cattle were stored for 24 h at 27±1°C, 20 days at 5°C and 60 days at –15°C. There was a proportionate increase in MFI as the post-mortem aging of meat advanced. It was concluded that the myofibrillar fragmentation followed a pattern of chicken > sheep > goat > cattle muscle on post-mortem storage at different temperatures.

Keywords : Myofibrillar fragmentation index, pH, Post-mortem, Aging, Chicken, Sheep, Cattle.

Myofibrillar proteins are largely responsible for rigor mortis and tenderness of meat. The structural changes occurring in skeletal muscle during post-mortem storage of carcasses at refrigerated temperature, leading to tenderization are thought to be due to proteolytic enzymes (Penny 1980; Dutson 1983; Goll et al. 1983; Koohmaraie 1988). The phenomenon of myofibrils breaking into shorter segments at or near the Z-line during post-mortem storage of muscle is termed as the myofibril fragmentation. It has been reported that myofibril fragmentation accounts for more than 50% of the variation in tenderness of steaks from the longissimus muscle of conventionally handled carcasses (Moller et al. 1973; Olson and Parrish 1977; Mac Bride and Parrish 1977). Olson and Parrish (1977) developed a technique for determining myofibril fragmentation of post-mortem muscle and termed the value as myofibrillar fragmentation index (MFI). The MFI is a measure of post-mortem proteolysis (Koohmaraie 1988) and it increases with post-mortem aging. The change in pH of muscle on post-mortem storage has also a vital role to play in its keeping quality (Beltran et al. 1997; O'Halloran et al. 1997; Silva et al. 1999). The changes of MFI characteristics in carcasses of animals immediately after slaughter are well-known (Koohmaraie et al. 1991; Whipple and Koohmaraie 1992). But, the changes with respect to storage of muscles at different temperatures and their relationship with pH have not been studied much. Normally, carcasses are stored in slaughter houses at low temperature until use. The present study was undertaken to evaluate the comparative changes in pH and myofibril fragmentation on storing the muscles of different species of animals at different temperatures.

Samples of longissimus dorsi muscle from cattle (beef), goat and sheep and leg muscles of chicken were separated from the carcasses within 30 min of slaughter, packed in low density polyethylene pouches and stored at 27±1°C for 24 h or 5°C for 20 days or –15°C for 60 days. The weights of cattle, goat, sheep and chicken at the time of slaughter ranged from 120-125 kg, 8-9 kg, 9-10 kg and 1.75-2.0 kg, respectively.

Finely minced muscle sample (5 g) was mixed with 20 ml of 150 mM KCl containing 5 mM sodium iodoacetate. homogenized for 15 sec and the pH of the homogenate was determined immediately.

Approximately 1 g of muscle sample was homogenized in a phosphate buffer (100 mM KCl, 20 mM K phosphate, 2 mM $MgCl_2$, 2 mM EDTA, 1 mM sodium azide, pH 6.8) centrifuged at 1000 x g for 10 min and the supernatant was discarded. The sediment was washed with salt buffer for four times and then precipitated with trichloroacetic acid (10% w/v). The precipitate was dissolved in 1 N NaOH by incubating in a water bath maintained at 37°C for 90 min. The protein content was estimated and the MFI was calculated as per the procedure described by Culler et al (1978).

The pHs of different muscles stored at 27±1°C for 24 h were found to vary from 6.68 to 6.97. The reduction in pH was more in chicken than other meats (Fig 1). The pHs of muscles were found to change from 6.68-6.97 to 5.28-5.39 at 27±1°C by 24 h post-mortem. Similar changes for cattle muscle were shown by Cassens and Newbold (1966) and Bodwell et al (1965). The present study showed that the decline of pH followed the pattern of chicken > cattle > goat > sheep on holding the muscles at 27±1°C.

The results depicted in Fig. 2, 3 and 4 indicated an increase in MFI for muscles with time after excision during storage at different temperatures irrespective of the species

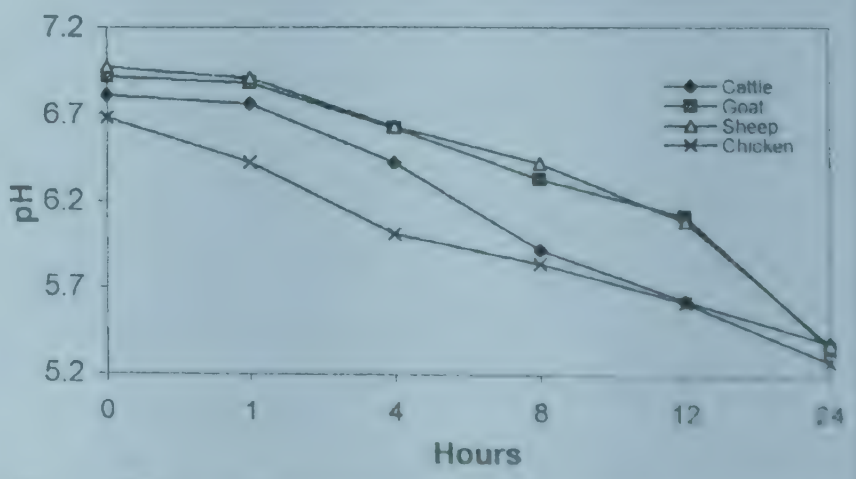

Fig. 1. Effect of post-mortem time on pH of different carcasses at 27±1°C

* *Corresponding Author*

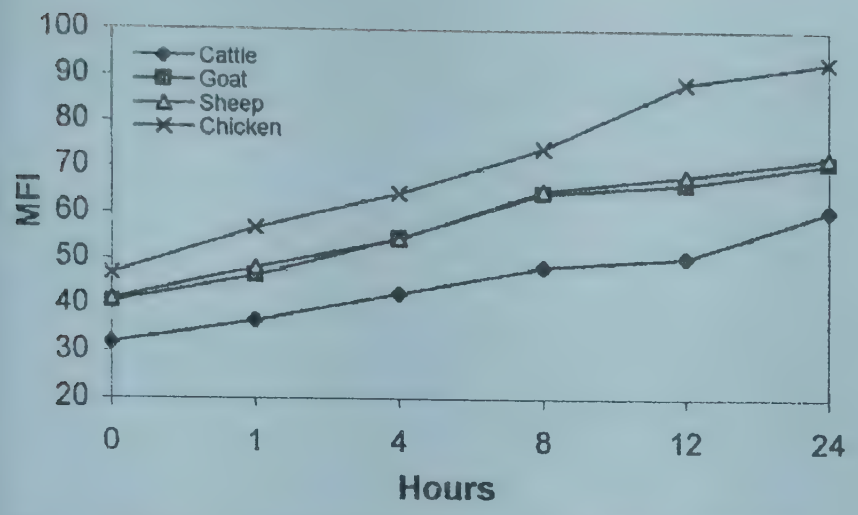

Fig. 2. Effect of post-mortem time on MFI of different carcasses at 27±1°C

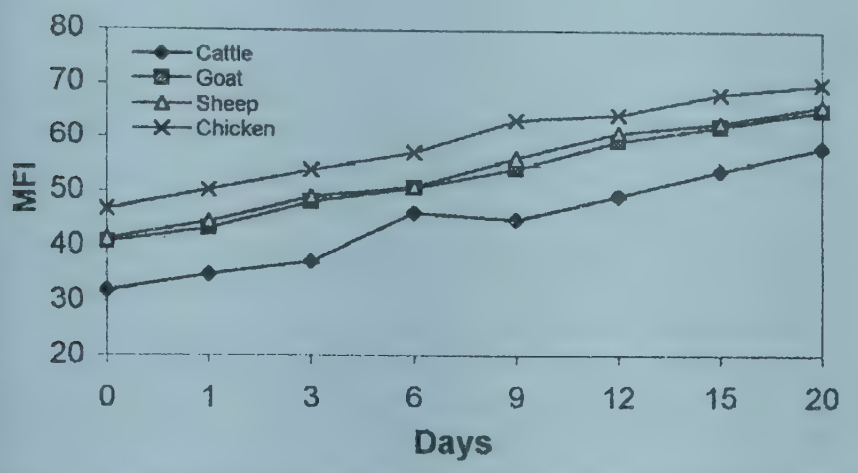

Fig. 3. Effect of post-mortem time on MFI of different carcasses at 5°C

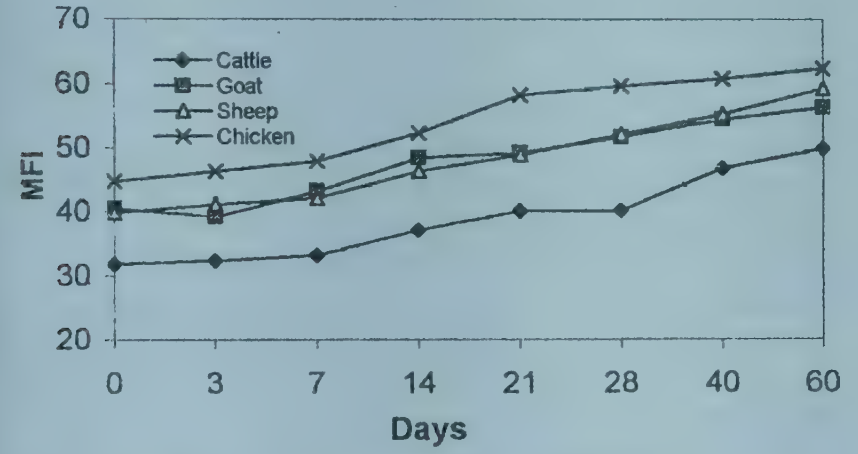

Fig. 4. Effect of post-mortem time on MFI of different carcasses at –15°C

studied. The fragmentation of myofibrillar proteins was faster in chicken over a period of 24 h. The initial MFI values were also higher in chicken as compared to other meats. At 0 h, the MFI values for muscles from different species of animals varied from 31.8 to 46.7. At 5°C (Fig. 3) or at –15°C (Fig. 4), the MFI was lower compared to the muscles stored at 27±1°C and lowest at –15°C. The MFI of cattle, sheep and goat stored at 5°C for three days and at –15°C for 14 days were comparable with that of the one kept for 1 h at 27±1°C. Chicken meat also showed similar results but at –15°C for 21 days was comparable to the one stored at 5°C for 6 days and for 1 h at 27±1°C.

The myofibrillar fragmentation followed a pattern of chicken > sheep > goat > cattle muscle on storage at different temperatures. These results are also important in determining the tenderness of steaks from the muscle. Further studies on the nature of proteolytc activity of degradation of myofibrillar proteins and the quality of tenderness are warranted to elucidate more information.

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Studies on Effect of Pre-treatments on Quality of Soy*paneer*

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Soymilk *paneer* was prepared by subjecting soybean to pre-treatments such as soaking in solution each of 1% $NaHCO_3$ and NaOH, cooking under pressure and boiling in distilled water for 30 min. The soaking pre-treatment in 1% solution of $NaHCO_3$ was found better in the contents of protein, fat, total solids and yield of *paneer*. The organoleptic qualities of this *paneer* were also observed to be superior over the *paneer* prepared by other pre-treatments, but inferior to buffalo milk *paneer*.

Keywords : Soymilk, Soy*paneer*, Soaking, Pressure cooking, Boiling, Organoleptic quality.

Soybean (*Glycine max* L.) is one of the most important legumes because of its high protein and fat contents and therefore it is being utilized for increasing the protein contents of many processed foods (including milk products). Among the coagulated milk products, soy*paneer* is being utilized on a large scale as a substitute to milk *paneer*. However, large variations in the acceptability of this soy*paneer* have been reported by a number of researchers. The unacceptability could have been largely because of flatulance causing oliogosaccharides and of beany flavours. Technologies to overcome these problems have also been developed by giving pre-treatments to the seeds. Badenhop and Hackler (1970), Parmar et al (1989), Ewan et al (1992), Chopra and Prasad (1994) recommended various pre-treatments such as soaking in salt solutions/water, pressure cooking, boiling etc. The organoleptic quality of soy*paneer* is affected by a number of factors one of them being the varieties of the raw materials used. For this study, the most promissing newly evolved variety 'Pooja' cultivated in this area was selected at random for the preparation of soy*paneer* and the data generated on the effect of various pre-treatments on the quality characteristics of the product are reported in this communication.

Seeds of soybean variety 'Pooja' were obtained from All India Coordinated Soybean Improvement Project. Marathwada Agricultural University. Parbhani. The seeds were cleaned and stored in airtight containers at ambient temperature (20-25°C) until use. The seeds were soaked in 0.05 N NaOH solution for 1 h, sun-dried and dehulled manually to get soy*dhal* as per the method described by Badenhop and Hackler (1970).

Pre-treatments and preparation of soymilk : Soy*dhal* (100 g) was soaked in 200 ml (1:2 w/v) 1% solution each of $NaHCO_3$ and NaOH for 12 h at room temperature and termed T_1 and T_2, respectively. The soaked water was discarded and *dhal* was washed for 2-3 times with water, which was drained off. Same quantity of *dhal* was partially cooked in 200 ml water (1:2 w/v using a pressure cooker (Marlex, 16 lit, Marlex Products Pvt. Ltd., Mumbai) till release of steam vapour within 2 min after the start of 1st whistle and termed T_3. One more lot of same quantity of soy*dhal* was boiled in 250 ml distilled water (1:2.5 w/v for 30 min and water was decanted as per the method of Parmar et al (1989) and termed T_4. All the pre-treated soy*dhal* samples were ground in Rallis mixer-cum-grinder with water of 85-95°C in the ratio of 1:10 w/v. Resulting suspensions were filtered through a double layered muslin cloth and the filtrates were boiled for 10 min with continuous stirring to prevent sticking of solids and scorching.

*Preparation of soy*paneer : The method of Nasim et al (1986) was adopted for *paneer* preparation. The soymilk obtained by adopting the various pre-treatments to soy*dhal* was heated to a coagulation temperature of 75°C using 1% citric acid solution. The coagulant was added with continuous stirring, till it coagulated and whey was removed by filtering through a double layered muslin cloth. The coagulum, thus obtained was pressed in cold water for 3 h after cutting into pieces of 2.5 x 5.0 x 2.5 cm size to help remove excess citric acid and stored at 5°C. The buffalo milk *paneer* prepared following the method of Bhattacharya et al (1971) was used for comparison. The protein (N x 6.25) and fat contents of soy*paneer* and buffalo milk *paneer* were determined by using standard AOAC (1980) methods. The data were analysed for standard significance as per the method of Panse and Sukhatme (1967). The organoleptic qualities of soy*paneer* prepared from different varieties of soybean milk and of buffalo milk after deep-fat-frying in vanaspati or hydrogenated vegetable oil were evaluated by using a modified score card, as suggested by Arora and Gupta (1991).

Data presented in Table 1, show that the pre-treatment T_1 gave higher contents of protein and fat, total solids and better yield of both soymilk and soy*paneer* than the rest of the treatments. However, there was no difference in protein contents between pre-treatments T_2 and T_3. The soy*paneer* contained more proteins than soymilk in all the pre-treatments, while the fat contents were low in T_4 and T_3. The reductions in the total solids and yield of both soymilk and soy*paneer* in pre-treatments T_3 and T_4 when compared to T_1 and T_2 were possibly due to losses of large quantities of materials at the time of filtration during the preparation of soymilk. The losses might have also been due to denaturation and hydration of proteins. Lower values of fat contents in pre-treatments T_3 and T_4 were due to losses fat and water holding capacity of *paneer* during coagulation. Similar results have also been reported by Parmar et al (1989). However, their differences in these parameters were non-significant in *paneer*. Lo et al (1968) also reported a decrease in the extraction of fat into soymilk when NaOH soak was employed and suggested that this was caused by a direct interference of diffusion of small molecules as well as larger protein molecules.

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TABLE 1. EFFECT OF PRE-TREATMENTS ON PHYSICOCHEMICAL CHARACTERISTICS OF SOYMILK AND SOY*PANEER*[a]

Pre-treatments	Soybean			Soymilk				Soy*paneer*			
	Proteins, %	Fat, %	Total solids, %	Proteins, %	Fat, %	Total solids, %	Yield, %	Proteins, %	Fat, %	Total solids, %	Yield %
Raw soybean ('Pooja')	39.46	20.17	89.28								
T_1 (soaked in 1% $NaHCO_3$ solution)	–	–	–	2.98	1.79	5.98	838.0	15.68	3.75	26.05	184.0
T_2 (soaked in 1% NaOH solution)	–	–	–	2.61	1.65	5.42	807.6	15.47	3.50	25.63	173.0
T_3 (pressure-cooked till first whistle)	–	–	–	2.59	1.34	4.95	781.0	15.45	3.40	25.47	154.0
T_4 (boiled in distilled water for 30 min)	–	–	–	2.56	1.31	4.94	775.0	15.42	3.43	25.47	153.0
	SE ±			0.03	0.02	0.02	2.09	0.01	0.03	0.05	1.47
	CD at 5%			0.10	0.06	NS	7.24	0.04	0.13	0.16	5.09

a - Each value is the average of four determinations

TABLE 2. EFFECT OF PRE-TREATMENTS ON ORGANOLEPTIC QUALITIES OF SOY*PANEER*[a]

Pre-treatments	Organoleptic qualities				
	Flavour (40)	Body and texture (30)	Appearance (10)	Colour (10)	Taste (10)
T_1	30.21	23.00	6.66	5.81	5.83
T_2	23.60	10.30	4.66	3.66	4.33
T_3	24.39	10.30	4.66	3.66	4.33
T_4	22.33	9.25	3.66	3.66	3.33
Buffalo milk *paneer*	34.44	25.00	7.66	7.33	7.33
SE ±	1.00	0.81	0.40	0.44	0.40
CD at 5%	3.45	2.82	1.41	1.52	1.41

T_1 - Soaked in 1% $NaHCO_3$ solution T_2 - Soaked in 1% NaOH solution

T_3 - Pressure cooked till first whistle T_4 - Boiled in distilled water for 30 min

a - Each value is the mean score of ten trials by 20 semi-trained judges

Organoleptic qualities : It is clear from the data presented in Table 2 that buffalo milk *paneer* scored maximum for all organoleptic qualities than *paneer* prepared from soybean with various pre-treatments. Among the pre-treatments, T_1 obtained significantly ($P<0.05$) better scores than T_2, T_3 and T_4. The significant differences were also observed among the various pre-treatments. The high organoleptic scores of pre-treatment T_1 could be possibly due to inhibition of undesirable beany flavour in the product. Babje et al (1992) and Ewam et al (1992) reported similar observations. The low organoleptic scores of *paneer* prepared by soaking soy*dhal* in 1% solution of NaOH were due to chalky texture, which could have resulted in the presence of the strong alkaline condition and this could have broken down the protein structure and reduced the guminess. whereas the *paneer* prepared from pressure cooking (T_3) and boiling (T_4) of soy*dhal* was brittle and grainy texture, which could be due to denaturation and hydration of the proteins. Parmar et al (1989) also made similar observations of tofu prepared after these pre-treatments.

It may be concluded that soaking pre-treatment in 1% solution of $NaHCO_3$ could reduce most of the undesirable beany flavour of soy flour and good quality *paneer* could be prepared by adopting this pre-treatments.

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RESEARCH NOTE

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Effect of Boiling on Beta-carotene Content of Forest Green Leafy Vegetables Consumed by Tribals of South India

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Thirty two edible forest green leafy vegetables (GLVs) usually consumed by the tribals of Andhra Pradesh, India were boiled according to their traditional boiling procedure and total carotenoids (TC) and beta-carotene (BC) contents were estimated. Per cent losses of TC and BC contents due to boiling ranged from 5.67 to 84.14 and 2.66 to 92.34 mg/100g, respectively. Wide variations in losses of beta-carotenes in the boiled GLVs were observed to be not proportional to cooking time.

Keywords : Edible forest foods, Carotenoids, Beta-carotene, Boiling losses, Tribals.

Vitamin A deficiency (VAD) has been known to be the underlying cause of xerophthalmia in many countries (Sommer and West 1996) and is recognised as a serious public health problem in India, as it contributes to considerable proportion of blindness in children. It is reported that 25% of the world total number (15 million blind) are from India of whom about a fifth can trace the origin to VAD. Food and Agricultural Organisation (FAO 1993) has recommended production and consumption of foods rich in vitamin A as the only truly sustainable solution to the problem.

Forests are a precious endowment of nature to mankind bountifully bearing omnifarious resources for sustenance of humans. The forest clad mountains and hills are the original habitats of hundreds of tribal groups in India, which are repository of nutritious food that grow wild and are neither cultivated nor marketed. There is no documented evidence for forest foods containing vitamin A. Several factors (i.e., genetics, environment of the plant, interplant, diurenals) influence the formation and concentration of carotenoids *vis-a-vis,* vitamin A activity. Tribals have the knowledge of edible forest foods to which they are habituated for generations.

There is often a lack of understanding about the dietary factors, which influence bioavailability as well as positive/negative effects that cooking and preservation techniques can have on the beta-carotene content of foods. Prolonged cooking was said to have deleterious effect on the retention of both beta-carotene and total carotenoids (Madhava Peddi et al. 1994). Cooking at elevated temperatures in the presence of oxygen is said to cause conversion of *all-trans* cerotene to *cis* isomers with decrease in vitamin A activity (Speek et al. 1988, Vanderpol et al. 1988). However, the losses in total carotenoids (TC) need not reflect the losses in beta-carotene. Most of the earlier studies have not touched this aspect. The present study, therefore, was undertaken to investigate the forest GLVs consumed by tribals and the effect of boiling on beta-carotene content.

Selection of area : Three districts namely Vizianagaram, Vishakapatnam and East Godavari from north east Andhra Pradesh, South India, where the tribal population is concentrated were selected for the study. The per cent forest areas to total geographical area in the three districts are 62.32, 74.5 and 49.42 respectively. The major tribals found in the area are 'Savara', 'Jatapu', 'Porja', 'Khonds', 'Bhagatha', 'Valmiki', and Konda Reddy'.

Procurement of foods : The sample collection was done during the months of July-October, as most of the GLVs are available during the period. The food samples were collected in quantities of 1-2 kg directly from forest with the assistance of the tribals of selected villages. The samples were sealed in polythene bags and carried to laboratory in an icebox and stored at –20°C till analysis. The GLVs were analysed within 24 h of their collection. Before analysis, all the GLVs were cleaned under running tap water and edible portion was used for the estimation of total carotenoids and beta-carotene.

Traditional method for boiling of GLVs : Tribals commonly practise boiling (with lid on) for cooking of the green leafy vegetables. The vegetable to water ratio generally used is 1:2. However, for 4 GLVs with which boiled water was drained off, the ratio used being 1:5 (Table 1). Leaves of GLVs were used as such instead of cutting to pieces as per the tribal practice and boiled in aluminium vessel (in the laboratory) over flame, till the leaves became tender. Before consumption, the cooked vegetables were mashed along with the remaining water and a little salt added to taste, or seasoned with small amounts of oil, chillis and onions as required or excess water was drained off after cooking, depending on the type of vegetable used (Table 1).

Analysis of the food samples : Total carotenoids and beta-carotene contents of both fresh and boiled GLVs were analysed using high performance liquid chromatography (HPLC), following the procedure as described by Nelis and Deleenher (1983).

Statistical analysis was done using one way ANOVA (Snedecor and Cochran 1965).

The contents of total carotenoids and beta-carotene content in fresh forest GLVs ranged from 6.39 to 35.63 and 0.95 to 14.12 mg/100g, respectively and the per cent loss of TC and BC due to boiling showed a wide range of 5.67 to 84.14% and 2.66 to 92.34% respectively (Table 1). Earlier reports have indicated that cooking with lid is more effective in retention of TC and BC content due to shorter time required for cooking and limited exposure to atmospheric oxygen (Jayarajan et al. 1980) However, in the present study, majority of the GLVs studied, showed 40-50% loss of BC. Cooking at elevated temperature

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TABLE 1. EFFECT OF BOILING ON TOTAL CAROTENOID AND BETA-CAROTENE CONTENTS OF FOREST GLVs COLLECTED AND CONSUMED BY THE TRIBALS

Green leafy vegetable		Carotenoids, mg/100g		Time taken for cooking, min	% loss on processing
		Unprocessed	Processed		
Gaju kura	TC	35.21	15.90	10	54.84
(Solanum nigrum)	BC	14.12	5.63		60.13
Mullu kura	TC	32.27	17.36	10	46.37
(Amaranthus spinosus)	BC	10.91	6.05		44.55
Pindi kura	TC	25.93	14.53	10	43.96
(Aerva lanata)	BC	10.81	3.36		68.92
Chipuru kura*	TC	35.63	5.65	20	84.14
(Cocculus sp.)	BC	10.28	1.95		81.03
Daggula kura	TC	32.70	16.11	10	50.73
(Amaranthus polygamus)	BC	9.12	5.18		43.20
Ponnaganti	TC	24.01	15.71	15	34.57
(Alternanthera sessilis)	BC	8.26	5.03		39.10
Adavi kanda	TC	30.13	8.73	10	71.03
(Amorphophallus sylvaticus)	BC	8.44	1.47		82.58
Silleru kura	TC	22.96	3.91	20	82.97
(Physalis minima)	BC	7.78	1.42		81.75
Nela benda	TC	25.74	13.76	15	46.54
(Hibiscus abelmoschus)	BC	7.78	4.29		44.86
Puvvu tota kura	TC	26.49	16.89	10	36.24
(Amaranthus viridis)	BC	7.18	3.56		50.42
Pilli Adugula kura	TC	25.83	13.97	10	45.92
(Oxalis corniculata)	BC	6.81	3.06		55.07
Ambali madu kura	TC	17.63	6.81	15	61.37
(Trianthema portulacastrum)	BC	6.40	1.17		81.72
Drumstick leaves*	TC	22.40	11.08	20	50.54
(Moringa oleifera)	BC	6.24	3.60		42.31
Sikaya chiguru	TC	27.00	15.40	10	42.96
(Acacia concinna)	BC	6.06	3.13		48.40
Tella tota kura	TC	24.72	20.17	10	18.41
(Amaranthus sp.)	BC	6.03	5.86		2.82
Pedda tantem kura*	TC	21.73	17.30	20	20.39
(Cassia occidentalis)	BC	6.02	5.86		2.66
Gurum kura	TC	12.64	10.55	15	16.53
(Celosia argentia)	BC	5.99	3.39		43.41
Podi kura	TC	16.44	13.01	15	20.86
(Lepidium sativum)	BC	5.79	3.30		43.00
White kura	TC	14.56	5.97	20	59.00
(Gynandropsis pentaphylla)	BC	5.77	3.12		45.93
Nagali sarekura	TC	18.57	8.80	15	52.61
(Allamania nodiflora)	BC	5.57	2.67		52.06
Amsukura	TC	27.35	10.87	15	60.26
(Adiantum punulatum)	BC	5.43	2.37		56.35
Chinna tantem* kura/Tentebu kura	TC	18.31	9.87	20	46.10
(Cassia tora)	BC	5.28	4.04		23.48
Garisa kura	TC	31.35	5.25	20	83.25
(Dryopteris cochleata)	BC	4.77	0.66		86.16
Adavi gummadi kura	TC	11.73	2.00	15	82.95
(Gmelina arborea)	BC	4.31	0.33		92.34
Gongura	TC	12.21	7.13	10	41.61
(Hibiscus cannabinus)	BC	3.60	2.65		26.39
Elukachevula kura	TC	17.95	5.14	15	71.36
(Convolvulus sp.)	BC	3.47	0.62		82.13
Venneduru kura	TC	13.54	7.20	10	46.82
(Commelina benghalensis)	BC	3.22	2.94		8.70
Bodantan kura	TC	9.19	5.09	15	44.61
(Bauhinia purpurea)	BC	2.94	1.51		51.36
Pulleru kura	TC	9.24	4.43	15	52.06
(Antidesma acidus)	BC	2.46	0.69		71.95
Chinnatummi kura	TC	6.39	2.17	15	65.00
(Leucas aspera)	BC	2.30	0.29		87.40
Simmadri kura	TC	6.70	6.32	15	5.67
(Alternanthera sessilis)	BC	2.26	0.82		63.72
chitti kura	TC	8.45	6.89	10	18.46
(Ficus cunia)	BC	0.95	0.82		13.68
Seshapkura	TC	28.67	0.96	20	18.46
(Globba bulbifera)	BC	5.55	–		10.0

TC : Total carotenoids; BC : Beta-carotene — TC SD ± 21.09
* Boiled and water drained off — BC SD ± 26.45

TC :
f calculated = 13280.32; SED = 0.258
CD (critical difference) 0.527 at 0.05% level of significance

BC :
f calculated = 15418.75; SED = 0.301
CD = 0.613 at 0.05% level of significance

in the presence of oxygen is reported to cause much losses of *trans* beta-carotenes (Sweeny and Marsh 1970) and trans to *cis* isomerisation (Vanderpol et al. 1988), resulting in reduction of vitamin A activity. As is evident from Table 1, boiling resulted in lowering of TC and beta-carotene. The time required for cooking the GLVs varied from 10 to 20 min. It is also evident from the same Table 1 that wide ranges in losses of TC and BC are not directly proportional to cooking time. The losses in BC in the GLVs cooked for 10, 15 and 20 min. ranged from 2.82 to 82.58; 39.10 to 92.34 and 2.66 to 100%, respectively. The GLVs which required draining off excess cooked water and took maximum time for cooking showed minimum to maximum loss of beta-carotene (Table 1).

Least loss (2.66-26.39%) of beta-carotene on boiling was observed in 5 GLVs namely, 'Peddatantemkura' *(Cassia auriculata)* (2.66%), 'Tellathotakura' *(Amaranthus* sp.) (2.82%), 'Vennedurukura' *(Commelina bengalensis)* (8.7%), 'Chittikura' *(Ficus cunia)* (13.68%) and 'Gongura' *(Hibiscus cannabinus)* (26.39%).

Maximum loss of beta-carotene (81-100%) on boiling was seen with 9 vegetables 'Chipurukura' *(Cocculus* sp.) (81.03%), 'Silleru kura' *(Physalis minima)* (81.75%), 'Ambalimadukura' *(Trianthima portulacastrum)* (81.72%), 'Elukachevulakura' *(Convolvullus* sp.) (82.13%), 'Adavikanda' *(Amorphallus* sp.) (82.58%), 'Garisekura' *(Dryopteris cochleata)* (86.16%), 'Chinnatummikura' *(Leucas aspera)* (87.40%), 'Adavigummadikura' *(Gmelina arborea)* (92.34%) and 'Seshapkura' *(Globba bulbifera)* (100%).

The remaining GLVs showed beta-carotene losses in the range of 39.10 to 71.95%. It is evident from Table 1 that 'Tellathotakura' (*Amaranthus* sp.), which required less time (10 min.) and 'Peddatantemkura' *(Cassia occidentalis)*, which took more time (20 min.) for cooking showed the least loss i.e., 2.82 and 2.62%, respectively and both the GLVs are good sources of beta-carotene having 6 and 5.28 mg per 100g, respectively. Similar observations were reported in some varieties of vegetables (Harris and Levenberg 1960).

The present findings have clearly revealed that the time of cooking and loss of beta-carotene are not directly proportional as reported earlier (Madhavapeddi et al. 1994). The extreme variations in losses of beta-carotene observed, might be attributed to either texture of leaves (course or tender) and/or the occurrence of carotenoids as complex with protein in plants (Sweeny and Marsh 1970) or conversion of *trans* to *cis* isomers during cooking, resulting in lowering of vitamin A activity (Sweeny and Marsh 1971).

In conclusion, the results of the present study suggest that unfamiliar, unconventional beta-carotene-rich forest GLVs should be produced and popularised in non-tribal areas for dietary diversity and for improving the vitamin A nutritional status of the population. Alternate cooking methods for better retention of beta-carotene may be tried for the GLVs, which showed maximum loss of beta-carotene due to boiling. The GLVs, which required elaborate cooking methods of boiling and draining off excess water may be further studied for the presence of antinutritional factors if any.

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RESEARCH NOTE

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Procces Optimisation for Lactic Acid Production from Whey Using *Lactobacillus* Strains

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Acid whey obtained during *paneer* preparation from cow's milk was used as substrate for lactic acid production. *Lactobacillus acidophilus* NCDC 15, *L, helveticus* NCDC 5 and *L. casei* NCDC19 were used along with yeast extract (0.5,1.0 and 1.5%), glucose (1.0, 2.0 and 3.0%), potato starch (5.0, 10.0 and 15%) and molasses (5.0, 10.0 and 15.0%) as growth supplements. *L. casei* NCDC 19 inoculated at 2% in whey enriched with molasses (10%) significantly ($P<0.05$) produced maximum lactic and (5.4%), pH (2.41) and minimum residual lactose (0.91) after 48 h. at 42 ± 1°C.

Keywords : Lactic acid, Whey, Growth supplements, *L. casei.*

Whey obtained as a by-product during the production of *paneer, chhana* and cheese is a highly nutritious fluid portion of milk. It contains about 10% of total milk proteins. It is also rich in water soluble vitamins, lactose and minerals. In India, presently, about 3 million tonnes of whey are produced annually, containing a total amount of 12 lakh tonnes of valuable milk in terms of nutrients. The worldwide production of fluid whey runs into millions of tonnes and yet methods to utilize this material are not well developed. One of the major problems faced by the dairy industry is to find proper disposal for whey because of its over production as against its utilization. It is, in fact, one of the under-utilized by-products of dairy industry with considerable impact on the economy and environment. The ecologists are very much concerned about the heavy burden of disposing whey, as its rich concentration makes it a costly pollutant in the national sewage systems and water bodies. The BOD values for cottage cheese whey range from 30,000 to 40,000 mg/l. A load of 1,000 gallons of raw whey discharge into a stream would require the dissolved oxygen in over 4,50,000 gallons of unpolluted water for its oxidation (Gillies 1974).

As per the opinion of experts, a more sensible approach for whey pollution control lies in its conversion to a dry stable material suitable as a by-product for either human or animal consumption or fermentation of whey to various value-added commercial products such as ethanol, single cell proteins, lactose, Baker's yeast, methane, β-galactosidase and various organic acids.

One of the most important products of whey fermentation is lactic acid. Commercially, the major applications of lactic acid and its compounds are as food additives i.e. as dough improvers, emulsifiers and plasticizers. Industrial applications of lactic acid in food industry are quite diverse. In view of above, the present investigation was undertaken to optimize the processing conditions for lactic acid production and its recovery from whey using *lactobacilli* strain and different additives such as molasses, potato starch, yeast extract and glucose.

Fresh cow's milk obtained from Livestock Research Center of this university was separated off cream, using an electric operated cream separator. Whey obtained by coagulation of separated milk using 2% citric acid solution at 70°C was used as substrate. The freeze-dried cultures of *Lactobacillus acidophilus* NCDC-15, *Lactobacillus casei* NCDC-19 and *Lactobacillus helveticus* NCDC-5 obtained from National Dairy Research Institute, Karnal, were activated in sterilized litmus milk at 37±1°C, then, maintained in sterilized litmus milk by fortnightly transfers and stored at 5°C between transfers. The OD values of 1, 2 and 4% inoculum were 1.2, 2.2 and 4.0 at 720 nm, respectively on of 10^{-1} dilution.

Glucose (1, 2 and 3%) yeast extract (0.5, 1.0 and 1.5%), molasses (5, 10 and 15%) and potato starch (5, 10 and 15%) were used as growth supplements. Titratable acidity (ISI: SP, 18, XII), pH, residual lactose ISI: SP-18, XI, calcium lactate and lactic acid were estimated according to the method of Ling (1951) with modifications as suggested by Harper and Randolph (1960).

Whey used in this investigation had 6.59% total solids, 5.42% lactose, 0.38% fat, 0.23% proteins and 0.56% ash.

Among cultures (*L.acidophilus* NCDC-15, *L.casei* NCDC-19 and *L.helveticus* NCDC-5) inoculated at 1, 2, and 4% showed significantly higher titratable acidity ($P<0.05$) in samples inoculated with *L. casei* NCDC-19 at 2% level at 42±1°C, as compared to 37±1°C after 48 h. of incubation.

Whey supplemented with glucose (1%) exhibited maximum acid production (2.47%) along with *L.casei* NCDC-19 (inoculum at 1%) in comparison to 2 and 3% glucose levels, whereas *L. acidophilus* NCDC-15 showed minimum acid production on 48 h. storage at 42±1°C. *L.casei* NCDC-19 showed maximum decrease in pH being minimum (3.08) and maximum (3.11) at 1 and 3% glucose levels, respectively when compared to other species of *lactobacilli.* It is apparent from Table 1 that pH decreased at much faster rate in *L.casei* NCDC-19, irrespective of glucose level. Residual lactose was minimum (1.12%) in whey samples inoculated with *L.casei*-NCDC-19 and fortified with 1% glucose, whereas higher levels of glucose (2 and 3%) showed highest residual lactose after 48 h. inoculation at 42±1°C. The present findings are in accordance with those reported by Gupta and Gandhi (1996). This may be indicative of the fact that glucose is not the limiting factor for growth of *lactobacillus.* The subsequent decline in lactic acid production of increasing glucose levels may be indicating the inhibitory role played by glucose on the production of lactic acid by either reducing the growth of

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TABLE 1 EFFECT OF LACTIC ACID CULTURES AND SUPPLEMENTS ON TITRATABLE ACIDITY, pH AND RESIDUAL LACTOSE DURING INCUBATION AT 42±1°C

Culture	Level of growth supplements	Titratable acidity			Incubation period, h			Residual lactose		
	Glucose, %	24	36	48	24	36	48	24	36	48
L. acidophillus NCDC 15	1	0.87	1.23	1.52	3.67	3.54	3.43	1.70	1.56	1.54
	2	0.83	1.20	1.40	3.68	3.55	3.48	1.71	1.57	1 54
	3	0.82	1.12	1.30	3.69	3.58	3.51	1.72	1.59	1.56
L. casei NCDC 19	1	0.90	1.82	2.47	3.66	3.32	3.08	1.63	1.42	1.21
	2	0.87	1.72	2.42	3.67	3.36	3.10	1.70	1.50	1.32
	3	0.84	1.69	2.40	3.68	3.67	3.11	1.72	1.50	1.32
L. helveticus NCDC 5	1	0.87	1.21	1.59	3.67	3.55	3.41	1.70	1.60	1.56
	2	0.87	1.21	1.50	3.67	3.55	3.44	1.70	1.57	1.54
	3	0.82	1.15	1.40	3.69	3.57	3.47	1.71	1.60	1.55
	Yeast extract, %									
L. acidophillus NCDC 15	0.5	1.04	1.72	1.90	3.60	3.36	3.29	1.62	1.50	1.41
	1.0	0.98	1.40	1.60	3.63	3.47	3.40	1.65	1.58	1.54
	1.5	0.90	1.19	1.32	3.66	3.55	3.51	1.70	1.60	1.58
L. casei NCDC 19	0.5	1.20	1.83	3.40	3.55	3.32	2.84	1.60	1.40	1.13
	1.0	1.03	1.60	3.30	3.61	3.40	2.85	1.64	1.50	1.14
	1.5	0.92	1.30	2.90	3.65	3.51	3.95	1.70	1.56	1.14
L. helveticus NCDC 5	0.5	0.99	1.60	1.90	3.63	3.40	3.29	1.60	1.57	1.41
	1.0	0.90	1.40	1.60	3.66	3.48	3.40	1.70	1.58	1.55
	1.5	0.89	1.10	1.50	3.66	3.59	3.44	1.71	1.15	1.56
	Molasses, %									
L. acidophillus NCDC 15	5	2.52	2.82	3.19	3.07	2.97	2.87	1.20	1.01	1.12
	10	4.21	4.90	5.20	2.61	2.49	2.41	1.06	1.06	0.98
	15	4.04	4.27	4.28	2.66	2.60	2.60	1.07	1.13	1.05
L. casei NCDC 19	5	3.1.	3.19	3.41	2.9	2.87	2.81	1.14	0.97	1.10
	10	4.3	4.75	5.4	2.59	2.5	2.41	1.05	1.05	0.91
	15	4.2	4.4	4.6	2.62	2.57	2.59	1.06	1.72	1.04
L. helveticus NCDC 5	5	1.88	1.99	2.10	3.30	3.26	3.20	1.44	1.40	1.40
	10	2.07	2.20	2.5	3.23	3.18	3.07	1.41	1.38	1.20
	15	2.0	2.05	2.2	3.24	3.24	3.18	1.93	1.50	1.35
	Potato starch, %									
L. acidophillus NCDC 15	5	1.30	1.70	2.95	3.51	3.36	2.94	1.60	1.50	1.15
	10	1.70	2.10	3.20	3.37	3.22	2.87	1.49	1.37	1.11
	15	1.80	2.30	3.40	3.33	3.15	2.82	1.44	1.33	1.12
L. casei NCDC 19	5	1.91	2.18	3.30	3.29	3.19	2.85	1.43	1.35	1.10
	10	2.04	2.30	3.70	3.24	3.15	2.74	1.38	1.32	1.09
	15	2.50	2.70	3.80	3.07	3.00	2.72	1.19	1.18	1.08
L. helveticus NCDC 5	5	0.98	0.99	1.10	3.63	3.63	3.59	1.62	1.61	1.58
	10	0.99	1.00	1.60	3.63	3.62	3.40	1.62	1.61	1.56
	15	1.00	1.70	1.78	3.62	3.37	3.34	1.61	1.50	1.45

Average of 3 trials

CD for titratable acidity, pH and residual lactose

	Titratable acidity	pH	Residual lactose
Between culture (I)	0.15	0.08	0.086
Between supplements (J)	0.16	0.09	0.093
Between supplement concentrations (K)	0.15	0.08	0.086
Between incubation period (L)	0.15	0.08	0.086
Between IJ	0.21	0.11	0.122
Between IK	0.20	0.11	0.114
Between IL	0.20	0.11	0.114

lactobacillus or its activity. The effect of *L.casei* NCDC-19 along with glucose supplement on titratable acidity, pH and residual lactose was significantly different (P<0.05) from other strains of *lactobacilli*. Different levels of glucose did not exhibit any significant effect on titratable acidity and pH on 48 h. storage at 42±1°C. However, residual lactose showed a significant effect.

Whey samples supplemented with yeast extract exhibited maximum acid production (3.4%) at 0.50% level with *L.casei* NCDC-19 in comparison with 1.0 (3.3%) and 1.5 (2.9%) levels whereas *L.acidophilus* NCDC 15 showed minimum titratable acidity after 48 h incubation at 42±1°C. *L.casei* NCDC-19 with yeast extract showed maximum decrease in pH being minimum (2.84) and maximum (2.95) at 0.5 and 1.5% levels. Decrease in pH was at a faster rate in *L.casei* NCDC-19 as compared to other species, irrespective of supplementation levels. Residual lactose level was minimum in *L.casei* NCDC-19 at 0.5% level of yeast extract. The present results are in accordance with the observations made by Norton et al (1994), who reported increase in lactic acid production by decreasing levels of yeast extract.

Whey fortified with molasses at the rates of 5, 10 and 15% showed significant difference (P<0.05) within the levels irrespective of *lactobacilli* strains. *L.casei* NCDC - 19 with 10% molasses produced significantly highest titratable acidity (5.4%) and lowest pH and residual lactose, respectively. The molasses used in this study had sucrose 35%, glucose 6.5% and fructose 8.5%.

Molasses and yeast extract are only growth supplements, which have less energetic importance. Experiments conducted with these growth supplements showed that the lowest concentration of these factors supported growth and their highest concentrations were found inhibitory. The present observations are in accordance with those of Gupta and Gandhi (1995).

Whey fortified with potato starch (5, 10 and 15%) showed significance difference (P<0.05) within the levels irrespective of lactobacilli strains. *L.casei* NCDC-19 with 15% potato starch produced significantly highest titratable acidity (3.80%) in comparison with 5% (3.3%) and 10% (3.70), whereas *Lactobacillus helveticus* NCDC-5 showed minimum titratable acidity in whey on 48 h. storage.

Lactobacillus casei NCDC-19 showed maximum decrease in pH being minimum (2.72) and maximum (2.85) at 5 and 15% potato starch levels with respect to other species of *lactobacillus*. It is apparent that pH decreased at much faster rate in *Lactobacillus casei* NCDC-19 as compared to other species of *lactobacillus*, irrespective of potato starch supplementation levels. Residual lactose was minimum (1.08%) in whey samples inoculated with *Lactobacillus casei* NCDC-19 and supplemented with 15% potato starch, whereas other supplementary levels (5 and 10%) showed higher residual lactose after 48 h incubation at 42 ± 1°C.

Potato starch is a source of energy for the growth of lactic acid bacteria. Potato starch is first degraded to glucose, which in turn, is broken down to lactic acid. The supply of glucose may be maintained at a steady rate. This statement can be supported by the fact that very high concentration of glucose suppresses lactic acid production. Hence, increasing potato starch concentration at higher levels enhanced the production of lactic acid because glucose is produced from starch at a steady rate so that the initial concentration of glucose in the medium supports prolific growth of *lactobacillus* and hence resulted in an increased production of lactic acid.

TABLE 2. EFFECT OF MOLASSES ON TITRATABLE ACIDITY CALCIUM LACTATE AND LACTIC ACID PRODUCTION ON 48 h STORAGE OF WHEY AT 42±1°C

Culture	Medium	Titratable acidity, %	Calcium lactate/ 100 ml	Lactic acid yield. g/100 ml
L. acidophillus NCDC 15	Whey + 10% molasses	5.20	2.03	4.86
L. acidophillus NCDC 15	Whey + 15% molasses	4.28	1.64	3.94
L. casei NCDC 19	Whey + 10% molasses	5.40	2.11	5.06
L. casei NCDC 19	Whey + 15% molasses	4.60	1.79	4.32
L. helveticus NCDC 5	Whey + 10% molasses	2.50	0.98	2.35
L. helveticus NCDC 5	Whey + 15% molasses	2.20	0.89	2.16

Average of 3 trials

The effect of molasses on titratable acidity, calcium lactate and lactic acid recovery on 48 h storage at 42 ± 1°C is shown in Table 2. *L.casei* NCDC 19 produced maximum titratable acidity (5.4%) in molasses enriched whey (10%), followed by *L.acidophillus* - NCDC 15 (5.2%) and minimum in *L.helveticus* NCDC 5 (2.5%). The amount of calcium lactate obtained was found directly related to the percent titratable acidity produced in whey. The calcium lactate yield (2.03 g/100 ml substrate) and lactic acid recovery (5.06%) were highest for *L.casei* NCDC 19 and lowest for *L. helveticus* (0.89 and 2.16, respectively). Amounts of supplements and cultures added to whey had a significant effect on per cent titratable acidity, calcium lactate yield and lactic acid recovery (P<0.05).

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Antioxidant Activity of Tropical Lemon Grass *(Cymbopogon citratus)* Extracts in Linoleic Acid and Chicken Fat Systems

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All extracts (dichloromethane and methanol extracts) and fractions (non-volatile and volatile) of lemon grass *(Cymbopogon citratus)* showed antioxidant activities in linoleic acid model system. However, in a more complex system, i.e., the chicken fat only the non-volatile fraction exhibited antioxidative activity. Lemon grass appeared to be a potential source of natural antioxidants, the active components of which could not be unidentified.

Keywords : Lemon grass *(Cymbopogon citratus)*, Linoleic acid model system, Chicken fat, Lipid oxidation, Spices, Peroxide values, Thiobarbituric acid-reactive substances (TBARs).

Concern about the safety of synthetic antioxidants together with consumer preference for natural products has aroused much research interest on natural antioxidants. Although rosemary has been the spice of major interest, the potential commercialisation of antioxidants based on other spices is receiving increasing attention (Madsen and Bertelsen 1995). Due to an increasing demand for new 'ethnic' foods, lemon grass (called *serai, xa or takrai)* has become one of the popular emerging ingredients (Uhl 1996). Several studies on the therapeutic properties of lemon grass and the antimicrobial activities of its essential oils have been carried out (Nakatani 1994). However, there have been only a few studies on the antioxidant properties of lemon grass (Baratta et al. 1998; Lim and Suhaila 1999). Lim and Suhalia (1999) observed that although the ethanol extract of lemon grass showed antioxidant property in butter-containing plain cakes, its activity was lower compared to turmeric, betel leaves and clove extracts. However, Baratta et al (1998) showed that the antioxidant activity of the essential oils of lemon grass was comparable to that of BHT and α-tocopherol. Since very little is known about the antioxidant activity and the antioxidant compounds of lemon grass, the aim of this work was to study the antioxidant activities of the various fractions of lemon grass extracts in a linoleic acid model and chicken fat systems in an attempt to isolate the fraction with active antioxidant compounds and also to determine, if it can be a likely potential source of natural antioxidant for foods.

Extraction : Extract of lemon grass was prepared according to the method of Kikuzaki and Nakatani (1994). Lemon grass, obtained locally, was dried (moisture content of ~11%), ground and extracted with dichloromethane. The residue obtained was subsequently extracted with methanol. The dichloromethane and methanol extracts were then concentrated to yield viscous residues. Steam distillation was carried out on the dichloromethane extract to produce the non-volatile and volatile fractions.

Antioxidant effects of lemon grass extracts/fractions in linoleic acid model system : Linoleic acid (90%) was purchased from Fluka, Switzerland. The linoleic acid model systems with added extracts/fractions of lemon grass, α-tocopherol and butylated hydroxytoluene (BHT) were prepared according to the method of Osawa and Namiki (1981). A mixture of sample (4 mg) in 4 ml 99.5% ethanol, 4.1 ml 2.51% linoleic acid in 99.5% ethanol, 8 ml 0.05M phosphate buffer (pH 7.0) and 3.9 ml distilled water were distributed in vials with screw caps. During storage in the dark at 50°C, duplicate vials were taken out at suitable time intervals for analyses. The peroxides and 2-thiobarbituric acid-reactive substances (TBARs) formed were determined by the ferric thiocyanate (FTC) method (Mitsuda et al. 1967), and the TBA method (Wilbur et al. 1949), respectively.

Antioxidant effect of lemon grass extracts / fractions in chicken fat system : Chicken fat was prepared according to the method of Kosugi and Kikugawa (1985). Fatty tissues of chicken were heated at 60°C for 5–6 h. The upper layer of chicken fat was collected and washed with hot distilled water.

The extracts and fractions of lemon grass, BHT and α-tocopherol were added to the chicken fat at a concentration of 0.02% (w/w). During storage, duplicate samples were taken out at suitable time intervals for the determination of PVs (AOCS 1985) and TBARs. The absorbance was measured at 532 nm (Fong et al. 1973).

In linoleic acid model system, low absorbance values obtained by both the FTC (absorbance at 488 nm) and TBA methods (absorbance at 532 nm) indicated a high level of antioxidant activity. Fig. 1 shows that all extracts and fractions of lemon grass have lower absorbance values at 488 nm than the control, with the non-volatile fraction having the lowest absorbance values during the storage at 50°C. The non-volatile fraction also produced a longer induction period (~1.5 days) than the other extracts/fractions (~1.0 days). The individual activities of these extracts and fractions measured on the third day using the TBA method are summarised and compared (Fig. 2). Similar patterns of activities were observed with the TBA method. The results showed that all extracts and fractions of lemon grass exhibited antioxidant activities, with the non-volatile fraction exhibiting the strongest activity. Based on the present index, it was interesting to note that the antioxidant activity of the dichloromethane extract was considerably better than α-tocopherol, but worse than BHT (Fig. 1). However, these

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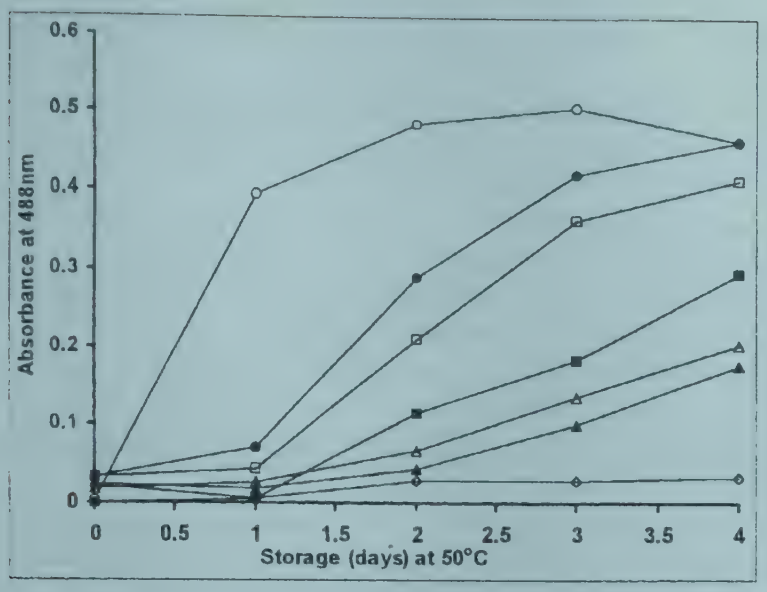

Fig. 1. Absorbance values of extract/fractions of lemon grass, α-tocopherol and BHT in linoleic acid model system using the FTC method. Each sample, conc. 0.02%. o-o control (without additives); Δ-Δ dichloromethane extract; ●-● volatile fraction, ▲-▲ non-fraction; □-□ methanol; ■-■ α-tocopherol; ◊-◊ BHT

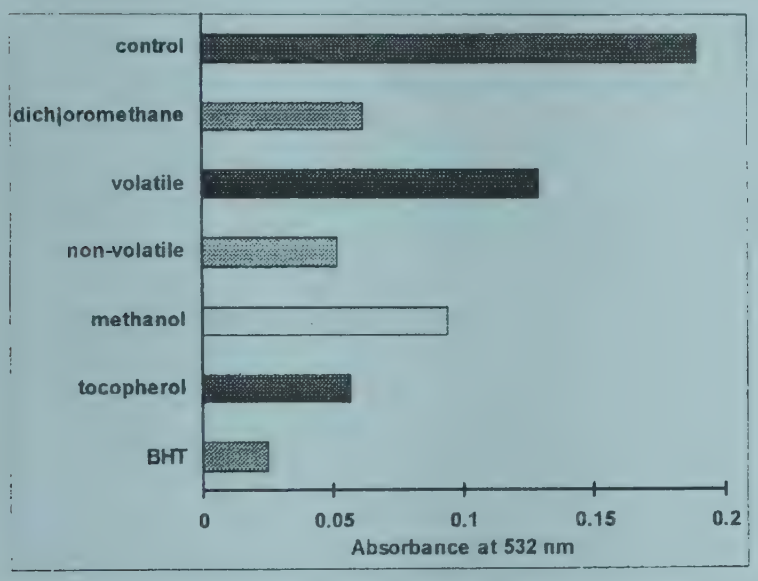

Fig. 2. Absorbance values (on 3rd day) of extract/fractions of lemon grass, α-tocopherol and BHT in linoleic acid model system using the TBA method. Each sample, conc. 0.02%

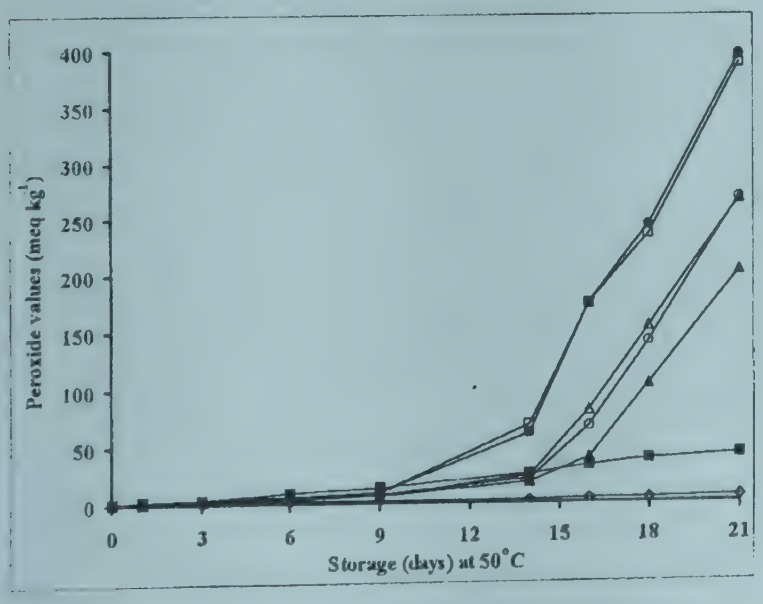

Fig. 3. Peroxide values of extract/fractions of lemon grass, α-tocopherol and BHT in chicken fat. Each sample, conc. 0.02%. o-o control (without additives); Δ-Δ dichloromethane extract; ●-● volatile fraction, ▲-▲ non-fraction; □-□ methanol; ■-■ α-tocopherol; ◊-◊ BHT

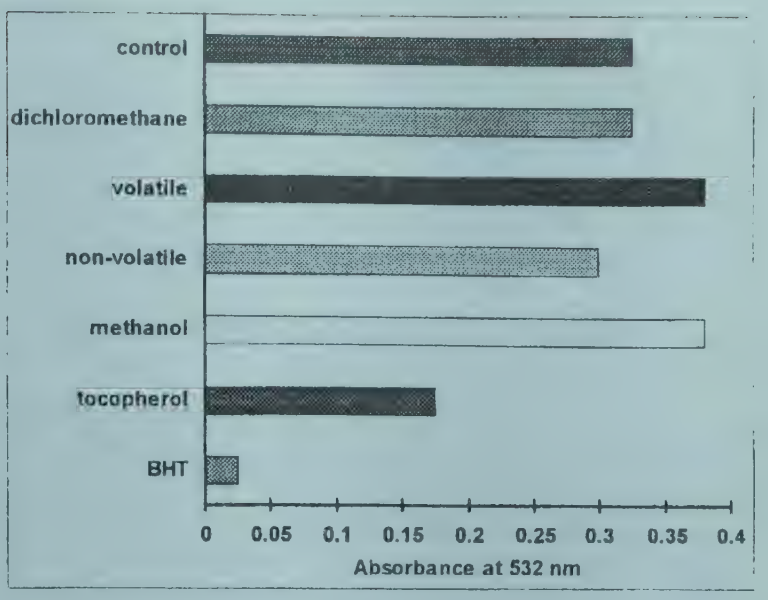

Fig. 4. Absorbance values (0n 21st day) of extract/fractions of lemon grass, α-tocopherol and BHT in chicken fat using the TBA method. Each sample, conc. 0.02%

differential antioxidative properties of dichloromethane extract and non-volatile fraction were not so clearly reflected as far as the production of secondary products was concerned (Fig. 2), suggesting that the antioxidant activity was primarily exerted during the initiation stage of lipid oxidation. More work needs to be carried out to elucidate the actual mechanism(s) involved.

In chicken fat, a different trend was observed. Both the methanol extract and the volatile fraction were found to give rise to higher PVs and TBARs than the control, indicating prooxidant activities (Fig. 3 and 4). Only the addition of non-volatile fraction resulted in lower PVs (Fig. 3) and TBARs (Fig. 4) than the controls, but higher PVs and TBARs than α-tocopherol and BHT. In a more complex system such as chicken fat, the non-volatile fraction still exhibited antioxidant activity which was, however, lower than that of the commercial antioxidants studied. The active antioxidant components that are likely to be present in the non-volatile fraction include the phenolic substances as many spices are known to contain phenolics, which exhibit antioxidant activities (Nakatani 1994; Adegoke et al. 1998). In fact, Faruq et al (1994) have reported that lemon grass oil possesses phenolics such as elemicin, catechol and hydroquinone. However, more work needs to be carried out to identify these active antioxidant compounds present in the non-volatile fraction. Although lemon grass is not as effective as commercial antioxidants in inhibiting lipid oxidation, it is a natural food ingredient and may be a potential source of natural antioxidants for food.

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RESEARCH NOTE

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Effect of Different Blanching Methods on the Physico-chemical Qualities of Frozen French Beans and Carrots

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Effects of low temperature long time (LTLT), high temperature short time (HTST) and mixed method (LTLT/HTST) on the physico-chemical qualities of carrots and french beans were investigated and compared with microwave blanching. Qualities of the vegetables blanched by mixed and microwave methods were found to be superior over those blanched by HTST and LTLT methods. Further, vegetables blanched by mixed and microwave methods showed least residual activity of peroxidase, high retention of ascorbic acid and total carotenoids, and better texture as compared to HTST and LTLT blanching.

Keywords : Blanching, Pectin methyl esterase, Microwave, Low temperature long time, High temperature short time.

Blanching is an important treatment, which primarily aims at inactivating the enzymes that cause undesirable changes during freezing preservation and subsequent storage of products. Optimum blanching under appropriate conditions of temperature and time conditions improves the quality and minimizes the disruptive changes. Over the years, application of low temperature long time (LTLT) as opposed to conventional high temperature short time (HTST) blanching in improving the quality has been emphasised. LTLT in comparison to HTST enhances the firmness and reduces the nutritional and flavour losses of the product (Lee et al. 1979; Taylor et al. 1981; Canet and Hill 1987; Seow et al. 1991). Mixed blanching involving a combination of LTLT and HTST or microwave with conventional blanching was found to give the best product from a total quality point of view (Dietrich et al. 1970). Peroxidase is the key enzyme responsible for off-flavour development and its inactivation is the primary function of blanching. Its inactivation secures better sensory and nutritional characteristics in frozen products. It is thus necessary that blanching conditions be optimized to have retention of nutritional characteristics and have low peroxidase activity.

The present study was aimed at studying the effects of conventional LTLT, HTST, mixed and microwave methods of blanching on the activity of peroxidase enzyme and physico-chemical quality of carrots and French beans.

Carrots *(Daucus carota)* and green French beans *(Phaseolus vulgaris)* were obtained from the local supplier, but the varieties were not known. The vegetables were thoroughly washed, sorted, hand-peeled (in case of carrots) and trimmed. Carrots were cut into 5 mm thick slices and French beans were cut into 10 mm French cut strips. The cut vegetables were separated into three lots and blanched accordingly.

Blanching methods : Conventional (HTST) blanching of the vegetables was done by immersing the vegetables in boiling water for 5 min in case of French beans and 3 min in case of carrots. Conventional LTLT blanching was done by immersing both the vegetables in water at 70°C for 30 min each. For mixed blanching (LTLT/HTST), the carrot slices were first blanched in water at 70°C for 30 min, followed by a second blanch in boiling water for 3 min. and microwave (MW) blanching was done in domestic BPL microwave (700W, 2450MHz) for 3 min for carrots and 4 min for French beans.

In all the treatments, the blanching water was maintained at neutral pH. After blanching, the material was immediately cooled under tap water, drained on a mesh tray for 10 min. and stored in a deep freezer (–18°C) for two months.

Analytical methods : The blanched samples were then analyzed for ascorbic acid content, peroxidase activity, drip loss and texture.

Ascorbic acid content was determined by titrating a known weight of sample against 2, 6 dichlorophenol indophenol dye using 3% metaphosphoric acid as extracting medium. (AOAC 1980) Total carotenoids were determined by the method as described by Ranganna (1977). Peroxidase POD (E.C. 1.11.1.7) activity was measured as the change in absorbance at 470 nm using guaiacol and H_2O_2 as substrate according to the method of Thomas et al (1981). One unit of enzyme activity corresponds to change in absorbance of 0.1/min. Drip loss of the material was determined by draining the material over a sieve for 5 min. and the drained weight was determined. The thawed samples were then transferred back to the polythene bags and re-frozen. The above procedure was repeated through five freeze thaw cycles and the drained weight was determined each time. The drip loss at the each freeze thaw cycles was then calculated. The firmness or the texture was measured subjectively by a panel of 10 judges on a 9-point Hedonic scale.

Experimental design and data analysis : The experiment was conducted in randomized design with repeat measures over time. All analyses were made in triplicates. Data were analyzed for standard error mean (SEm) (Snedecor and Cochran (1987).

Peroxidase enzyme is mainly associated with colour and flavour changes occurring during frozen storage of vegetables. Therefore, destruction of this enzyme during blanching is very important, as its presence is detrmintal to the quality of the frozen vegetables. However, complete destruction of this enzyme is also not desirable, as it leads to loss in the activities of

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TABLE 1 EFFECT OF DIFFERENT BLANCHING METHODS ON THE PHYSICO-CHEMICAL QUALITY OF CARROTS AND FRENCH BEANS

	Ascorbic acid, mg/100g f.wt		Total carotenoids, µg/100g f.wt		Peroxidase activity units/g f.wt	
	Carrots	French beans	Carrots	French beans	Carrots	French beans
Control	5.0 ± 0.05	26.0 ± 0.34	9760 ± 310	145.0 ± 6.0	12.0 ± 0.11	60.2 ± 4.1
LTLT	2.1 ± 0.03	11.0 ± 0.18	6150 ± 219	92.0 ± 7.0	5.1 ± 0.03	32.4 ± 3.2
HTST	1.4 ± 0.01	9.0 ± 0.12	4850 ± 177	72.0 ± 4.0	2.6 ± 0.02	25.5 ± 1.4
MW	3.9 ± 0.04	20.0 ± 0.26	8590 ± 292	115.0 ± 5.0	1.25 ± 0.01	10.2 ± 0.9
MIXED (LTLT/HTST)	3.7 ± 0.05	18.0 ± 0.18	8630 ± 302	110.0 ± 7.0	1.75 ± 0.01	13.1 ± 0.89

Each value is the mean of four replicates ± S.E.

several other enzymes, which otherwise are responsible for maintaining the quality of blanched products. Therefore, it is necessary to optimize the blanching conditions by taking into consideration all the parameters. In the present study, peroxidase activities of fresh French beans and carrots were found to be 60 units and 12 units/g on fresh weight basis respectively (Table 1). Blanching of carrots resulted in a maximum loss (~90%) in POD activity in MW blanching, followed by mixed (85%), HTST (80%) and LTLT (58%). Similar trend was observed in the case of French beans. The results indicated that MW blanching accounted for the minimum residual activity of peroxidase, which had positive implications in maintaining the quality of frozen vegetables (Steinburch 1991). This could be due to rapid heating in MW, resulting in faster inactivation of peroxidase as compared to conventional processing (Gavanthorn et al. 1991; Ramaswamy and Fakhouri 1998).

Ascorbic acid is generally used as an indicative component of the quality of processed food products because of its high degree of water solubility and heat lability. Average ascorbic acid contents of fresh unblanched carrots and French beans were found to be 5 mg and 26 mg/100 g on fresh weight basis. MW blanching of French beans accounted for maximum ascorbic acid retention (76%), while mixed blanching, LTLT and HTST showed 69%, 42% and 34%, respectively. Similar trend was observed in carrots, where MW blanching again showed the maximum retention of ascorbic acid. Higher losses observed in HTST method may be attributed to leaching losses due to high temperature. Similar results have been reported by Guines and Bayindirh (1993).

Total carotenoids, like ascorbic acid are also susceptible to oxidative loss caused by heat and light. Higher retention of total carotenoids was again observed in both MW and mixed blanching methods being 88% in carrots and 76-79% in French beans. HTST and LTLT methods accounted for substantial losses in total cartenoids in both the vegetables (Table 1). Chen and Han (1990) have also shown that in water convolvulus, conventional cooking had lower carotenoid content than MW cooking.

Better quality of the vegetable as exhibited by mixed (LTLT/HTHT) blanched material may be attributed to the better cell wall integrity through the firming effect of LTLT in two stage blanching, accounting for the better ability to withstand the disruptive influence of HTST (Rahman et al. 1971; Quenzer and Burns 1981). Reduced cooking time because of faster heating and less cooking water required for MW blanching probably contributed to retention of better quality products.

Texture is one of the most important quality attributes, affecting the acceptability of frozen fruits and vegetables. Excessive softness and mushiness in some cases and a tendency to slough are the major problems of frozen products subsequent to thawing.

Texture of the vegetables as revealed by the texture scores was highest in LTLT - blanched vegetables (8.0) and lowest in HTST method (Fig 1). Similar results have been reported by Seow et al (1991). This firming effect of mild heat treatment is attributed to activation of enzyme pectin methyl esterase (PME), which enhances the firmness of cell vegetables (Wu and Chang 1990). There was no significant difference between the textural scores of vegetables blanched by microwave and mixed methods. However, the values were nearly the same as observed in the case of LTLT method. It has been earlier shown by Stone and Young (1985) that texture of green beans blanched by microwave was better than that of the conventional methods (boiling water and steam).

The results of freeze-thaw stability are depicted in Fig 2 and 3. Drip loss was used to measure the freeze thaw stability. The greater the drip loss, the poorer was the stability. The drip

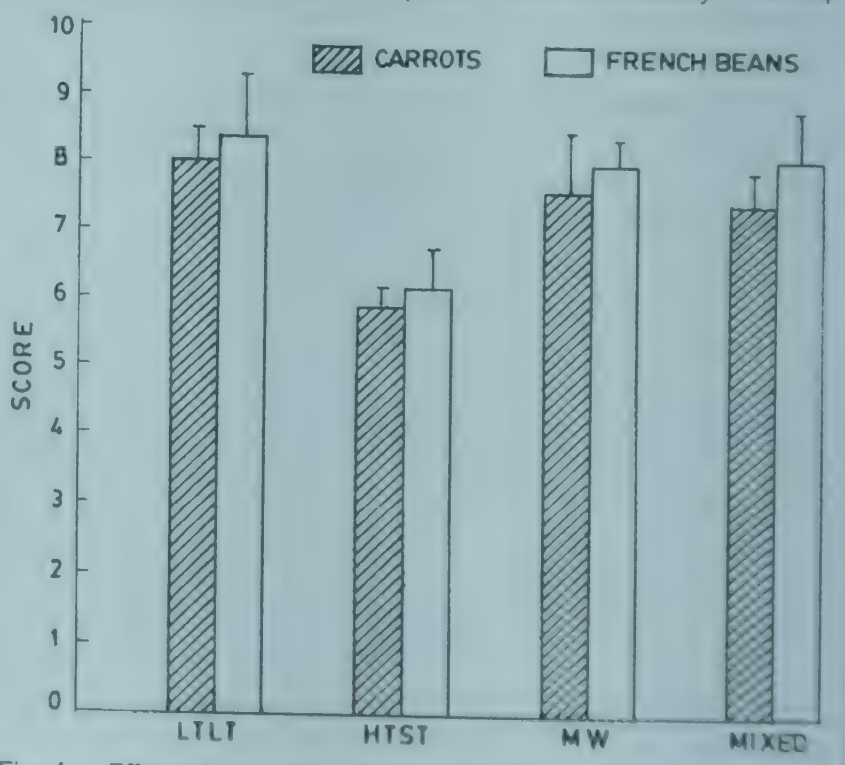

Fig. 1. Effect of blanching methods on the texture profile of French beans and carrots.

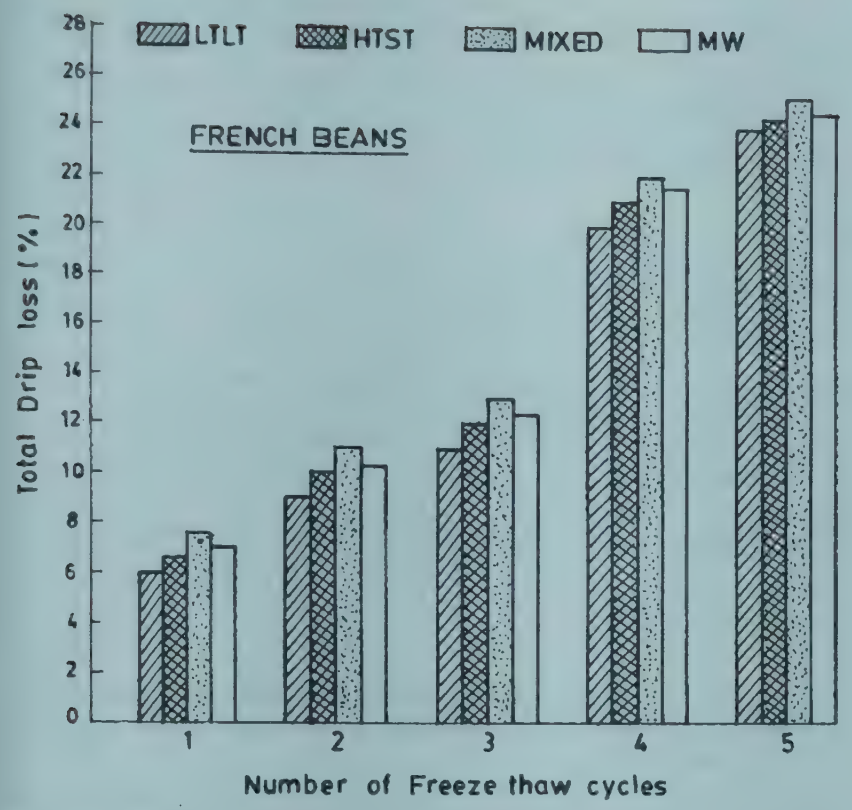

Fig. 2. Effect of freeze-thaw cycle on the drip loss in French beans.

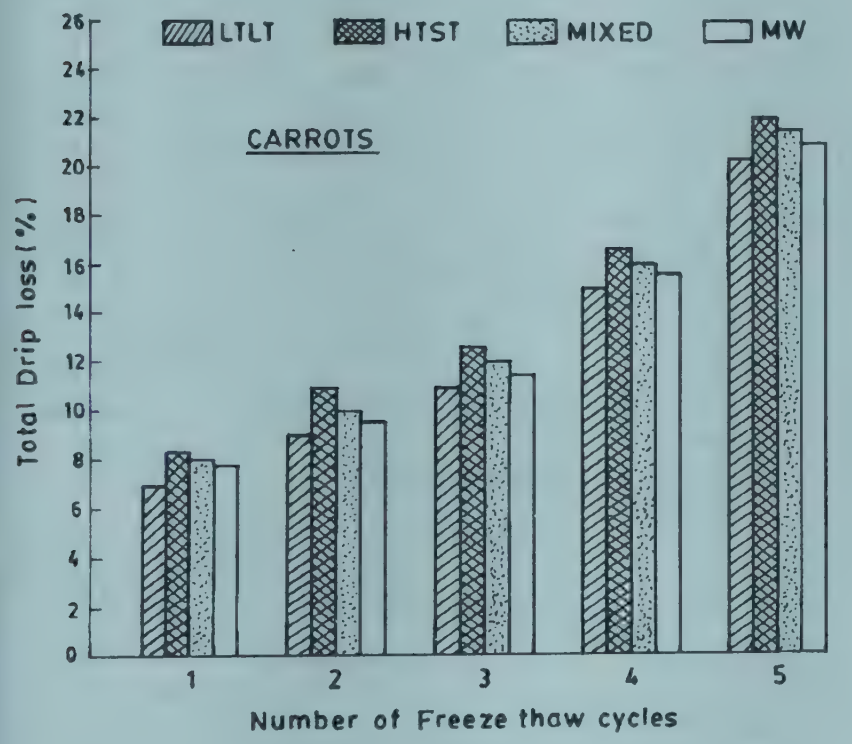

Fig. 3. Effect of freeze-thaw cycle on the drip loss in carrots.

loss increased with freeze-thaw cycle, but no significant difference was noticed between the freeze-thaw stability of the blanched treatments. Normally, it is expected that a firmer texture would improve the resistance to damage of the materials subjected to repeated freezing and thawing, thereby limiting the fluid loos from the tissues.

It may be concluded from this study that quality of vegetables blanched by mixed and microwave methods was superior to those of HTST and LTLT methods.

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Application of HACCP System for the Production of Edible Grade Soymeal

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Status of residual hexane in soymeal from different solvent extraction plants showed that the residual hexane levels ranged from 35-77 ppm. Total bacterial and fungal counts ranged from 6.9 to 8.0 x 10^3 and 7.9 x 8.5 x 10^3/g. respectively The meal contained 50-52% proteins with 15-22 NSI. Based on these results, application of Hazard Analysis Critical Control Point (HACCP) has been proposed to improve the quality of soymeal with the following critical control points viz., soybeans (CCP 1) and other unit operations like dehulling (CCP 2), steaming (CCP 3), flaking (CCP 4), solvent extraction (CCP 5), desolventization (CCP 6), grinding (CCP 7) and storage (CCP 8) of the meal. The seriousness of the hazards was then considered and checkups were suggested to control them to yield a good quality edible grade soymeal.

Keywords : HACCP, Critical controls, Soymeal, Residual hexane, Nitrogen solubility index.

Soybean oil industry produces about 3.0 million tonnes of soybean meal containing about 50-52% proteins annually. More than 80% of soymeal is exported to other countries, where it is primarily used for animal/poultry/piggery feed, thereby depriving the Indians of the good quality protein. The main bottleneck in direct food uses of soymeal is the presence of residual n-hexane, which might cause certain physiological disorders in humans. In order to find out the status of residual hexane in soymeal from different solvent extraction plants in Madhya Pradesh and assess its quality, the present studies were conducted to suggest a HACCP system for the production of edible grade soymeal from the existing solvent extraction plants. The present communication describes the quality controls and quality assurances for making an edible grade soymeal.

Five solvent extraction plants in and around Indore and Dewas (MP) were selected for the study. These representative samples were collected from each plant, which conformed to the Bureau of Indian Standards (1992). The samples were analyzed for crude proteins, crude fat, NSI, residual hexane and total microbial load using AOAC (1989) methods. For the total viable counts, the samples were serially diluted and aliquots of each dilutions were placed on suitable culture medium. For total bacterial counts, nutrient agar medium was used and for total fungal count, potatoe dextrose agar medium was used. The plate count method was used to enumerate the total viable counts. All the analyses were done in triplicate and the average values were expressed on as is basis. The residual hexane was detected using the over headspace technique developed at this Institute.

The status on the quality of soymeal from the five different solvent extraction plants is assessed initially and presented in Table 1.

The data indicated that most of the soymeal samples contained microbial load. The Nitrogen Solubility Index values were also lower. The residual hexane ranged between 35.2 ppm and 76.5 ppm. For edible purposes, the quality of the soymeal needs further improvements with reference to microbial counts, Nitrogen Solubility Index and residual solvent. This

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TABLE 1. CHEMICAL AND MICROBIOLOGICAL QUALITY OF SOYMEAL

Solvent extraction plant	Crude protein, %	Crude fat, %	NSI	Residual hexane, ppm	Total microbial load, x 10^3	
					Bacterial	Fungal
A	49.7	0.5	22.0	76.5	7.0	8.2
B	51.0	0.2	18.0	40.3	7.8	8.5
C	52.7	0.3	20.0	48.0	8.0	8.5
D	50.5	0.5	21.7	35.2	6.9	7.9
E	52.0	0.1	15.0	68.0	7.1	8.1

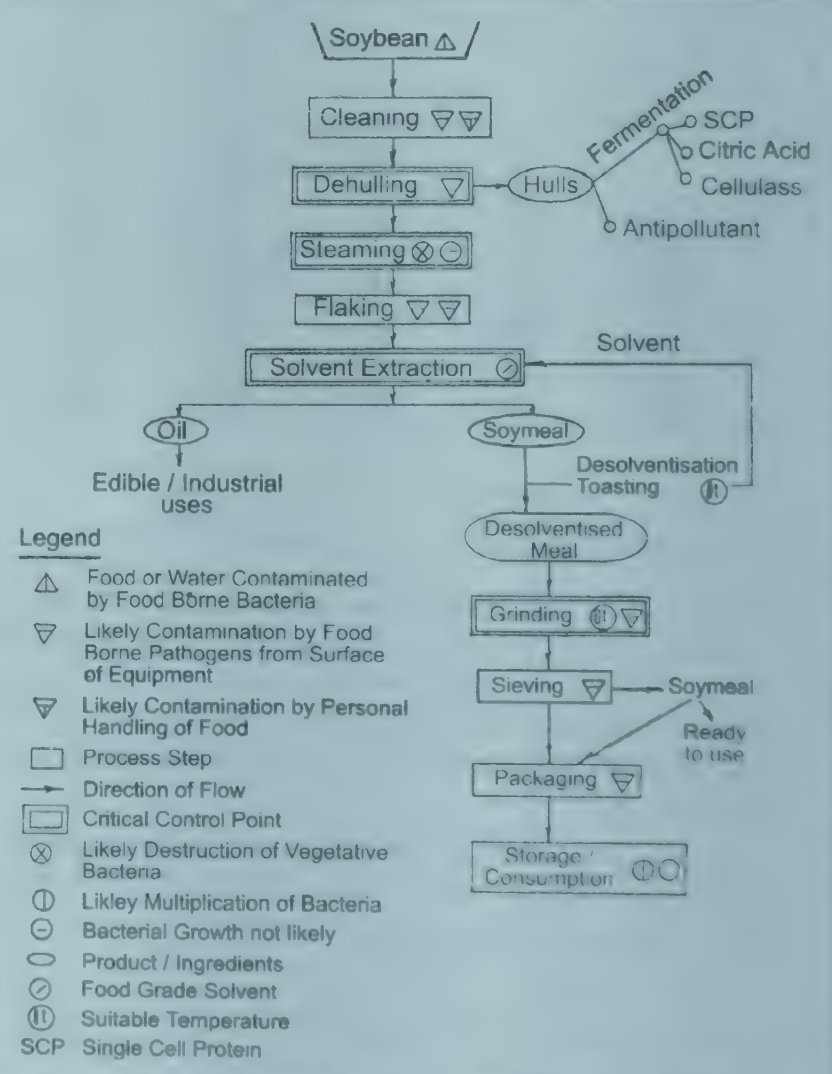

Fig. 1. HACCP flow sheet for the preparation of edible grade soymeal

TABLE 2. HACCP PLAN AND CONTROL CHART FOR MAKING EDIBLE GRADE SOYMEAL

HACCP plan					HACCP control chart			
Ingredients/ Processing g step	CCP No	Hazard	Preventive measure	Critical limits	Monitoring		Corrective action	Responsibility
					Procedure	Frequency		
Raw material (soybeans)	1	Contamination with field borne pathogens, foreign matter, dirt, stubbles and intrinsic factors like pesticide residues, aflatoxin etc.	Effective cleaning and processing methods	Legal limits as per BIS	Visual and testing in-house	Every batch	Select good source, store under hygienic conditions	Purchase Manager
Hulls	2	Microbial contamination	Dehulling	Total microbial load 50000/g	Microbial testing in-house	Every batch	Select a good dehuller	Production Operator
Water	3	Source for microbes and pollutants	Steaming	Legal limits of BIS	Testing of water for microbes and pollutants	Every batch	Select good source of water, Boiler with perfect controls	Production Operator
Flaking	4	Contamination through personal handing and surface of the equipment	Proper handling and correct choice of the flaker	Free from the contaminants and suitable for food use	Review the metallurgy of the flaker and personal hygiene	Every batch	Change the flaker and use protective clothing to operators	Quality Assurance Manager
Solvent extraction	5	Impurities of the solvent	Proper solvent	BIS limits for food grade solvent	Quality testing in-house	Every batch	Change the source	Quality Assurance Manager
Desolventi zation	6	Severe toasting	Optimum temperature and time	BIS limits for NSI, residual solvent and microbial load	Quality testing in-house	Every batch	Change the operational parameters	Production Operator/ Quality Assurance Manager
Grinding	7	Temperature rise during grinding and personal hygiene	Good controls	As per BIS	Quality test in-house and observe personal hygiene	Every batch	Replace the milling system and provide hygienic conditions	Quality Assurance Manager
Storage	8	Microbial contamination	Good packaging system and storage conditions	As per BIS, total microbial counts max 50000/g	Quality in-house	Every batch	Use a better packaging system and storage conditions	Quality Assurance Manager

could be possibly achieved by adopting HACCP system. (Mortimore and Wallace, 1994; Morris, 1997; Colon 1998).

For identification of sources of potential contaminations like microbiological, physical and chemical, a process flow chart for making soymeal was drawn and presented in Fig. 1. All the possible steps were noted and the hazard points and critical controls were identified. The major ones were soybeans (raw material), and the unit operations involved in the process namely dehulling, steaming, flaking, solvent extraction, desolventization, grinding and storage. The details of HACCP plan and controls are given in Table 2. HACCP system is regarded as a general approach to solving problem as well as an effective means of ensuring product safety. It is a very useful tool to identify the hazards and risks throughout the process. All the identified critical control points are to be under control to get a better quality edible grade soymeal. At each stage of processing, the product has to be evaluated for its quality characters.

A trial run was made for making edible grade soymeal with and without HACCP. The quality characteristics of the soymeal in both the cases are shown in Table 3.

TABLE 3. QUALITY CHARACTERS OF SOYMEAL WITH AND WITHOUT APPLICATION OF HACCP

Parameter	HACCP	
	Without	With
Crude proteins, %	51.0	51.0
Crude fat, %	0.9	0.9
NSI	25	65
Residual hexane, ppm	100	12
Colour	Dark	Pale
Total bacterial counts, per g	7.0×10^4	6.5×10^3
Total fungal counts, per g	8.0×10^3	7.0×10^2
Salmonella, no/g	Nil	Nil
Coliforms, no/g	5	Nil

Thus, results of these the studies have indicated that the quality of soymeal can be improved to a considerable extent

through the application of HACCP. This product has high NSI with negligible residual solvent. Though the cost of production was not computed, it implied extra expenditure. This extra expenditure will reflect in the better quality soymeal, which gets more premium.

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RESEARCH NOTE

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Malting Potential of Husked Barley in Relation to Proanthocyanidins

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Malting quality of two and six-row husked barley (12) grown under Haryana agroclimatic conditions were explored. Two-row barley varieties had higher thousand kernel weight, kernel plumpness, germination energy, germination capacity, malt friability, malt homogeneity and hot water extract, whereas proanthocyanidins, proteins, starch and husk contents were significantly higher in six-row barley. Proanthocyanidins in barley grains varied from 3.85 to 4.94 mg g^{-1} and none of the barleys was free from proanthocyanidin. Malting significantly decreased the starch and proanthocyanidins but increased the soluble wort nitrogen and Kolbach index. Considering all the quality traits, varieties 'Alfa 93', two-row and 'RD 2560', six-row emerged superior for malting and brewing purposes.

Keywords : Barley (*Hordeum vulgare* L.), Malting quality, Proanthocyanidins, Germination capacity, Tannin-protein interaction.

Barley (*Hordeum vulgare* L.) is an important cereal crop grown throughout world. About 10% of the world's barley production is utilized by malting and brewing industries and the remaining for human food and animal feed in different parts of the world (Lawrence 1988). Malt quality depends on barley quality that is influenced by biotic and abiotic factors (Fastnaught et al. 1996; Molina-Cano et al. 1997). The presence of proanthocyanidins in barley grains invites special attention of the brewing industry to exclude undesirable haze formation in wort and beer. Haze formation can be avoided by the use of proanthocyanidin-free barleys but such lines are low yielding and possess low diastatic power (Wesenberg et al. 1989; Horsley et al. 1991). Horsley et al (1995) indicated that proanthocyanidin-free cultivars with acceptable agronomic performance and malt extract could be developed by crossing.

This communication deals with malting quality of two and six-row husked barley grown under agroclimatic conditions in Haryana with particular reference to proanthocyanidins.

Barley (*Hordeum vulgare* L.) grains of two and six-row husked varieties 'BH 469', 'Alfa 93', 'UBE 467', 'UBE 469', 'UBE 991', 'BCU 73', 'BH 393', 'BH 85', 'RD 2503', 'RD 2560', 'K 551' and 'UBE 868' grown during the rabi season of 1997-98 were procured from the Department of Plant Breeding, CCS Haryana Agricultural University, Hisar. The grains were cleaned, air-dried, ground through a cyclotec sample mill (Tecator, AB; Hoganas, Sweden) fitted with 0.5 mm screen to deliver particle size of 0.45 mm and subsequently stored in airtight plastic containers at room temperature.

One hundred plumped grains of each variety were malted in an automatic micro-malting machine (Seeger Maschinenfabrik, Feilback, Germany). The dried malt was crushed lightly to remove rootlets and the malt yield was calculated (ASBC 1992).

Thousand kernel weight (TKW), kernel plumpness, malt friability, homogeneity, hot water extract (wort), wort colour and moisture contents were determined as per ASBC (1992). Kernels retained on sieves having a size of <2.2 mm and >2.5 mm were considered as thin and plump, respectively. Germination energy and germination capacity were worked out by the method of Kent-Jones and Amos (1967). Sodium hypochlorite reagent was used for the determination of husk content of grain (ASBC) 1992).

Proanthocyanidins in native and malt samples were extracted in methanol and estimated using the vanillin-HCl method (Wettstein et al. 1977). Starch content was estimated after removing the soluble sugars by dissolution in 80% ethanol, followed by centrifugation and digesting the residue with 52% perchloric acid to its glucose residues (McCready et al. 1950) and subsequent reaction of glucose with phenol-sulphuric acid (Dubois et al. 1956). Protein was calculated from nitrogen content determined by the conventional micro-Kjeldahl method using a conversion factor of 6.25 (AOAC 1995). Soluble wort nitrogen was estimated according to the method of Vickery et al (1935). Kolbach index was calculated by dividing the soluble wort nitrogen by total nitrogen. All determinations were carried out in triplicate and data were analysed statistically (Snedecor and Cochran 1987).

Data presented in Table 1 indicate the TKW of two-row (40.0 to 56.3 g) and six-row (40.3 to 43.2 g) husked barley. Similarly, kernel plumpness of two-row ranged widely from 77.13 to 95.87% and of six-row barley from 69.63 to 95.03%. Barley varieties having higher TKW and plumpy kernels may be desirable for malting because of their greater malt extract (Mather et al. 1997). However, malt yield of two-row barley (85.07-88.78%) was slightly lower (Table 2) than six-row barley (87.78-90.53%).

Germination energy and capacity varied from 97 to 100% and from 95 to 99% in two-row husked varieties, respectively, while in six-row varieties, germination energy varied from 95 to 98% and germination capacity from 94 to 97%. All the varieties under study possessed good germination capacity and germination energy. However, germination energy was slightly higher than germination capacity, indicating that the varieties were water sensitive (Table 1). Similar observation was made by Sood et al (1987).

Moisture contents of the barley varieties ranged from 7.7 to 9.6%, whereas those of malt varied from 6.9 to 11.3% (Table 1). All the barley genotypes had proanthocyanidins and their contents varied from 3.85 to 4.94 mg g^{-1} on dry weight basis. Jende-Strid (1993) also reported values for

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proanthocyanidins, ranging from 0 to 3.15 mg g^{-1} dry weight. It is interesting to note that, the variety 'RD 2560' (six-row) contained the highest concentration (4.94 mg g^{-1}) of proanthocyanidins while its malt contained the minimum level of 2.43 mg g^{-1}. The mean proanthocyanidin contents of two and six-row barley varieties were, however, more or less same (Table 1). The increased polymerization of proanthocyanidins with the proteins and polyphenol oxidase activity could be the

TABLE 1. PHYSIO-CHEMICAL CHARACTERISTICS OF GRAIN AND MALT OF TWO AND SIX-ROW HUSKED BARLEYS (VALUES ARE MEAN OF THREE DETERMINATIONS)

Varieties	TKW, g	Kernel plumpness, %		Germination		Husk*, %	Moisture, %		Proanthocyanidins, mg g^{-1}		Protein, % dry wt.		Starch, % dry wt	
		Bold	Thin	Energy, %	Capacity, %		Grain	Malt	Grain	Malt	Grain	Malt	Grain	Malt
Two-row														
'BH 469'	51.3	95.87	2.33	99	97	9.70	8.9	9.7	3.85	2.71	11.15	10.51	47.34	37.61
'Alfa 93'	40.0	84.10	4.20	100	99	9.11	8.8	8.6	4.54	2.93	10.93	10.06	55.80	43.64
'UBE 467'	40.0	81.27	2.27	97	97	9.20	9.0	7.4	4.51	3.37	10.92	10.02	54.57	40.98
'UBE 469'	41.0	77.13	5.70	99	97	10.10	8.8	7.7	4.62	3.44	8.09	8.62	55.62	40.67
'UBE 991'	40.4	88.23	3.03	100	98	8.75	8.9	7.9	4.29	3.17	9.18	8.75	49.41	32.47
'BCU 73'	56.3	89.20	1.80	98	96	10.35	8.5	8.4	4.25	2.95	9.62	9.20	54.30	37.86
Mean	44.8	85.97	3.22	99	97	9.54	8.8	8.3	4.34	3.10	9.98	9.53	52.84	38.87
Six-row														
'BH 393'	41.3	83.60	3.73	95	97	12.64	7.7	8.1	4.76	3.11	10.06	9.61	56.70	40.53
'BH 85'	40.3	69.63	8.63	98	96	11.17	9.0	11.3	4.58	2.66	10.09	9.67	57.72	40.92
'RD 2503'	43.2	95.03	0.67	97	94	10.88	9.0	6.9	4.43	3.03	11.59	10.80	53.13	41.70
'RD 2560'	42.6	91.93	1.57	96	97	10.82	8.6	10.8	4.94	2.43	10.73	10.42	57.63	44.73
'K 551'	42.6	73.33	7.67	96	97	11.83	9.6	10.0	4.58	2.76	11.27	11.20	54.99	41.75
'UBE 868'	42.8	92.50	1.03	97	96	12.37	9.2	8.6	4.80	2.97	9.19	9.63	49.23	42.31
Mean	42.1	84.34	3.88	97	96	11.62	8.9	9.3	4.68	2.83	10.49	10.22	54.90	41.99
C.D. at 5%	0.62	6.83	1.70	NS	NS	1.73	NS	0.29	0.54	0.69	0.67	0.05	1.81	3.03

Bold>2.5 mm; Thin <2.2 mm
NS = Non-significant
*Grain only

TABLE 2. MALT AND WORT QUALITY CHARACTERISTICS OF TWO AND SIX-ROW HUSKED BARLEYS (VALUES ARE MEAN OF THREE DETERMINATIONS)

Varieties	Malt yield, % dry wt	Malt friability, % dry wt	Malt homogeneity, % dry wt	Hot water extract, % dry wt	Wort colour °E.B.C.	Wort appearance	Filtration rate, ml/h	Soluble-N, %	Kolbach index
Two-row									
'BH 469'	88.78	90	98	79.9	3.5-4.0	BR	300	0.58	0.35
'Alfa 93'	85.67	50	80	76.7	2.5-3.5	BR	282	0.66	0.41
'UBE 467'	85.07	73	93	78.3	2.5-3.0	BR	295	0.64	0.40
'UBE 469'	86.61	76	94	77.3	2.5-3.5	BR	212	0.66	0.48
'UBE 991'	85.97	82	96	79.0	2.5-3.0	BR	313	0.54	0.38
'BCU 73'	87.10	72	92	78.7	3.0-4.5	BR	265	0.62	0.42
Mean	86.53	73.83	92.17	78.3	–	–	278	0.62	0.41
Six-row									
'BH 393'	88.53	72	95	78.3	4.5-5.0	SHZ	185	0.56	0.36
'BH 85'	90.31	52	86	75.7	4.0-4.5	BR	303	0.49	0.32
'RD 2503'	87.78	64	83	77.4	3.5	BR	225	0.45	0.26
'RD 2560'	88.92	50	80	75.3	3.5	SHZ	268	0.59	0.35
'K 551'	87.88	48	88	79.2	3.5	BR	300	0.46	0.26
'UBE 868'	90.53	65	80	78.0	3.5-4.0	SHZ	271	0.40	0.26
Mean	88.99	59	85	77.3	–	–	259	0.49	0.30
C.D. at 5%	NS	9.16	7.40	NS	–	–	13.50	NS	NS

NS = Non-significant
°E.B.C. - European Brewing Conversion
BR = Bright wort colour
SHZ = Slightly hazy

possible reasons for the observed decrease in proanthocyanidin content in the malt (Yadav et al. 2000).

It is now well established that grain high in protein is undesirable, as it is associated with lower malt extract and it also poses problems with beer stability and viscosity. The present varieties, except 'UBE 469' were found to meet the recommended level of protein (Anon 1996). The possible reasons for reduction in protein content during malting could be partly due to its association with the proanthocyanidins and increased activity of proteolytic enzymes.

Starch is the major constituent of barley endosperm, which produces sugars at the time of malting and later forms a part of the wort. The high value of starch in barley grains consequently results in better wort. The starch contents in barley grains vary from 47.34 to 57.72% (Table 1) and decreased during malting, the values varying from 32.47 to 44.73%. Six-row barley had higher starch content than two-row varieties. The observed decrease in starch content during malting is in agreement with the findings of Subramanian et al (1992). Husk contents varied from 8.75 to 12.64% with maximum and minimum in 'BH 393' and 'UBE 991', respectively. More or less similar values have been reported by Gupta (1997). The husk contents of six-row varieties were significantly higher than two-row types.

Hot water extract is a key malting quality indicator because it reflects the amount of beer that can be produced from a given quantity of malt and it varied over a narrow range of 75.3 ('RD 2560') to 79.9 ('BH 469')% (Table 2). Malt friability ranged from 50 to 90% and from 48 to 72%, while malt homogeneity varied from 80 to 98% and from 80 to 95% in two and six-row barley, respectively. Compared to two-row barley, slightly higher values for wort colour were observed in six-row barley (Table 2). The wort of three, six-row varieties viz., 'BH 393', 'RD 2560' and 'UBE 868' had slightly hazy appearance because of the presence of higher levels of proanthocyanidins, while the remaining nine varieties had bright appearance. The lowest filtration rate in 'BH 393' may be due to the slight hazy appearance of the wort and higher °E.B.C. units and / or due to higher amount of β-glucan, which led to high viscosity during mashing. Insoluble β-glucan plugs the filter bed and brings the filtration process to a stop, leading to further loss of malt extract. Similar views were also shared by Mather et al (1997). Two-row barley gave higher filtration rate than six-row barley. Soluble wort nitrogen ranged from 0.54 ('UBE 991') to 0.66% ('UBE 469' and 'Alfa 93') and from 0.40 ('UBE 868') to 0.59% ('RD 2560') with mean content of 0.62 and 0.49% in two and six-row barleys (Table 2). Kolbach index represents the percentage of malt nitrogen that is soluble and indicates total proteolytic activity during malting and mashing. These values varied widely and significantly from 0.26 ('K 551', 'RD 2503' and 'UBE 868') to 0.48 ('UBE 469'), Przulj et al (1998) also observed genotypic differences in Kolbach index in winter barley.

The above results revealed that a good variability existed among the barley varieties for almost all the parameters. This variation might be under the influence of genetic make up of cultivars and abiotic factors (Fastnaught et al. 1996; Molina-Cano et al. 1997; Przvlj et al. 1998). But the differences observed in the present study appeared to be mainly genetic ones as all the varieties were grown under identical agroclimatic conditions. The concentration of proanthocyanidins and starch decreased significantly, while soluble wort nitrogen and Kolbach index increased during malting. Taking majority of parameters into consideration, 'Alfa 93' among two-row and 'RD 2560' among six-row appeared to be superior for malting and brewing purposes.

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RESEARCH NOTE

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Studies on Use of Isabgol *(Psyllium mucilloid)* Husk in *Atta* for *Chapati* Making

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A study was conducted with the aim of increasing fibre content after incorporation of isabgol husk at the levels of 0.5, 1.0 1.5, 2.0, 2.5, 3.0 in *atta* for use in *chapati* making. Farinograph and pasting characteristics improved, as the level of fibre was increased. Mixing time of the flour decreased and dough became sticky in handling, as the flour absorbed more of moisture with increase in level of fibre. Puffing of *chapatis* was partial but browning was even. *Chapatis* made with flour containing isabgol were found acceptable by the panelists at all the levels of supplementation.

Keywords : Isabgol husk, Dietary fibre, Farinograph characteristics, Pasting properties, *Chapatis*.

The role of dietary fibre in controlling chronic disorders like diverticulitis, bowel cancer, cardiovascular diseases, diabetes, constipation etc. has been well documented (Painter and Burkitt 1975). Further, in particular, a diet rich in fibre, which contains plant cell wall is protective against a wide range of diseases. Among the plant cell wall, *Psyllium mucilloid* (isabgol) is accepted as a natural fibre and is used commercially in the pharmaceutical and other allied industries. In India, about 85% of the wheat product is consumed in the form of *chapatis* (unleavened flat bread) by a large segment of population of Indian sub-continent (Sidhu et al. 1989).

Chapatis are important sources of dietary proteins, calories, some of the vitamins, minerals and fibre. The present study was conducted by incorporating isabgol husk at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0% levels in *atta* from two wheat varieties. Effects of incorporation of isabgol husk at different levels on farinograph, amylograph and *chapati* making characteristics of *atta* were studied.

Procurement and preparation of samples : Two varieties of wheat 'PBW-138' and 'PBW-222' were obtained from the Department of Plant Breeding, Punjab Agricultural University, Ludhiana. The samples were cleaned thoroughly before milling.

Cleaned wheat samples were milled into whole wheat flour in a local stone *chakki*. The milled flour was sifted to remove the coarse bran (about 5%). It was stored in metallic bins for subsequent use in this investigation.

Psyllium mucilloid husk was procured from the local market and ground into fine powder in a Brabender Junior mill and was incorporated into *atta* 0.0, 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0% at levels.

Quality characteristics of wheat grains : Wheat grains of the varieties 'PBW-138' and 'PBW-222' were analyzed for various physico-chemical characteristics like thousand kernel weight, bulk density, hectolitre weight, grain density, proteins, ash, fat by standard AACC (1990) methods. Pearling index was determined by pearling a known weight of wheat sample in barley pearler for 45 sec and then calculating the percent overs.

Chemical composition of flour and fibre : Atta prepared from both the varieties of wheat were analyzed for proteins, fat, ash, damaged starch, diastatic activity, reducing sugars, non-reducing sugars, total sugars, gluten, and dietary fibre according to standard AACC (1990) and AOAC (1990) procedures.

Dietary fibre was analyzed by standard AOAC (1990) method. All other parameters were determined, using standard AACC (1990) methods.

Rheological properties of flour : Effect of fibre addition on the farinograph characteristics and pasting properties were studied according to standard AACC (1990) methods.

Preparation of chapatis : *Chapatis* were prepared by following the procedure of Sidhu et al (1989).

The sensory scoring of *chapatis* was done by semi-trained panelists on a 6-point scale. The results were statistically analysed using ANOVA and Duncan's multiple range test (Duncan 1955).

Quality characteristics of grains : The important quality parameters of grains of both the varieties are given in Table 1. Thousand kernel weight of 'PBW-222' was 45.69 g as against a value of 43.8 g for 'PBW-138'. Variety 'PBW-138' had higher protein and ash contents of 10.28% and 1.95%, respectively as compared to 'PBW-222' showing values of 10.05% and 1.65%, respectively but the difference was non-significant. Fat content of 'PBW-222' was 2.24% as against a value of 1.98% for 'PBW 138'.

Composition of Atta : Composition of *atta* is given in Table 2. *Atta* from 'PBW-138' had higher protein of 9.52, damaged

TABLE 1. PHYSICO-CHEMICAL COMPOSITION OF WHEAT VARIETIES

Parameter	Variety	
	'PBW-138'	'PBW-222'
*1000 kernel weight, g	43.80	45.69
Bulk density, g/cc	0.75	0.74
Hectolitre weight, kg/hectolitre	79.50	78.16
Pearling index, % overs	51.40	67.80
Grain density, g/ml	1.33	1.46
Proteins, %	10.28	10.05
Ash, %	1.95	1.65
*Fat, %	1.98	2.24

*Values vary significantly at p CD = 0.05

* *Corresponding Author*

starch content of 10.37 and gluten content of 8.89% than *atta* from 'PBW-222' with the values of 9.23%, 8.46% and 8.47% for proteins, damaged starch and gluten contents, respectively. *Atta* from 'PBW-222' had higher fat, ash, and total sugar contents with values of 1.91%, 1.63% and 12.66%, respectively than *atta* from 'PBW-138' with values of 1.69%, 1.50% and 11.79%, respectively.

Composition of isabgol : isabgol husk was analyzed for proteins, ash, fat, and dietary fibre and results are given in Table 2. It contained very high amounts of ash (2.14%) and dietary fibre (80%).

Effect of isabgol addition on farinograph characteristics : The farinograph characteristics of the dough viz; water absorption, dough development time, stability time, mixing tolerance index, degree of softening were studied and results are presented in Table 3. Farinograph water absorption increased with increase in level of supplementation. The water absorption for 'PBW-138' increased from 65.5 for control to 80.3 at 3% level of supplementation and similarly, for 'PBW-222', the value increased from 72.3% for control to 84.6% at 3% level of supplementation. Krishnan et al (1987) have also reported similar findings. Fibre's higher capacity of imbibing water is the reason for this increase.

Dough development time increased from 3.40 min. to 4.0 min. for 'PBW-138' and 4.10 min. to 4.90 min. for 'PBW-222' at 3% level of supplementation. Chen et al (1988) reported an increase in dough development time with increase in level of apple pomace that was used as a source of fibre and concluded that this increase could be the slower rates of hydration and development of gluten.

Stability time and mixing tolerance index showed significant improvements with increases in the levels of *isabgol* (Table 3). Stability time increased from 1.6 min for control to 1.9 min. for *atta* from 'PBW-138' and for *atta* from 'PBW-222', the value increased from 0.80 min to 1.9 min. at 3% level of supplementation.

The values for mixing tolerance index decreased from 62.5 BU for control to 15 BU for *atta* from 'PBW-138' at 3% level of supplementation. Similarly, the values of *atta* from 'PBW-222' decreased from 83 BU for control to 53 BU at 3% level of supplementation. Chen et al (1988) reported that dough stability increased with increase in level of fibre as fibre provided greater strength to gluten and made it resistant to mechanical damage.

Effect of isabgol addition on pasting properties of flour : The values for mean gelatinization time decreased from 19.8 min. for control to 8.3 min. at 3% level of supplementation for *atta* from 'PBW-138'. Similarly, for *atta* from 'PBW-222' the value decreased from 20.3 min. for control to 12.3 min. at 3%

TABLE 2. PROXIMATE COMPOSITION OF *ATTA* AND *ISABGOL* HUSK (14% MOISTURE BASIS)

Parameter	Variety		
	'PBW-138'	'PBW-222'	Isabgol
Proteins, %	9.52	9.23	0.91
Fat, %	1.69	1.91	0.35
Ash, %	1.50	1.63	2.14
Damaged starch, mg. maltose/10g	10.37	8.46	N.D.
Reducing sugars, % glucose	125.60	104.00	N.D.
Non-reducing sugars, % sucrose	3.94	4.87	N.D.
Total sugars, % glucose	11.79	12.66	N.D.
Gluten, % dry	8.89	8.47	N.D.
Dietary fibre, %	2.20	2.20	80

N.D. - Denotes 'not determined'.

TABLE 3. EFFECT OF ISABGOL HUSK *(PSYLLIUM MUCILLOID)* ON FARINOGRAPH AND PASTING CHARACTERISTICS OF *ATTA*

Characteristic	Variety	Level of supplementation							
		0.0	0.5	1.0	1.5	2.0	2.5	3.0	LSD
FWA, %	'PBW-138'	65.50	67.10	69.60	70.30	75.10	77.25	80.25	7.16
	'PBW-222'	72.25	75.75	76.60	78.10	83.30	83.60	84.60	
DDT, min	'PBW-138'	3.40	3.50	3.60	3.90	4.20	4.10	4.30	0.66
	'PBW'-222'	4.10	4.40	4.50	4.10	4.60	4.60	4.90	
Stability time, min.	'PBW-138'	1.60	1.60	1.50	1.40	1.60	1.70	1.90	0.71
	'PBW'-222'	0.80	1.20	1.00	1.20	1.40	1.30	1.90	
MTI, BU	'PBW-138'	62.50	45.00	57.50	77.50	58.50	58.00	15.00	51.13
	'PBW'-222'	83.00	60.00	73.00	73.00	78.00	78.00	53.00	
Gelatinization time, min.	'PBW-138'	19.80	16.30	16.80	14.30	11.80	11.30	8.30	5.28
	'PBW'-222'	20.30	18.30	18.40	16.30	15.10	14.30	12.30	
Gelatinization	'PBW-138'	64.30	60.30	57.30	56.30	53.30	51.30	47.30	2.74
	'PBW'-222'	65.80	62.30	61.80	58.80	56.80	56.30	53.30	
Temperature, °C	'PBW-138'	462.00	630.00	842.50	982.50	1030.00	1125.00	1375.00	
Peak, veiscosity, BU	'PBW'-222'	457.50	805.00	947.50	1025.00	1210.00	1337.50	1355.00	398.48

FWA - Farinographic water absorption
DDT - Dough development time
MTI - Mixing tolerence index
LSD - Least significant difference

level of supplementation. The *atta* from 'PBW-138' had more of damaged starch due to lower gelatinization time as compared to *atta* from 'PBW-222'.

The gelatinization temperature decreased significantly for both varieties. In case of *atta* from 'PBW-138', it decreased from 64.8°C for control to 48.3°C at 3% level of supplementation. Similarly, in case of *atta* from 'PBW-222', the values decreased from 63.3°C for control to 47.3°C at 3% level of supplementation.

TABLE 4. EFFECT OF ISABGOL HUSK ADDITION ON *CHAPATI* MAKING QUALITY OF *ATTA*

Variety	Level of addition	Water absorption %	Mixing time, min	Dough handling	Puffing	Browning
'PBW-138'	0.0	73.0	3.0	Smooth	Full	Uneven
	0.5	81.0	2.5	Smooth	Partial	Slightly even
	1.0	86.0	2.5	Smooth	Partial	Slightly even
	1.5	89.0	2.0	Smooth	Partial	Slightly even
	2.0	89.0	2.0	Slightly sticky	Partial	Even
	2.5	90.0	2.0	Slightly sticky	Partial	Even
	3.0	91.0	2.0	Slightly sticky	Partial	Even
'PBW-222'	0.0	75.0	2.5	Smooth	Full	Uneven
	0.5	78.0	2.5	Smooth	Partial	Slightly uneven
	1.0	80.0	2.0	Smooth	Partial	Even
	1.5	81.0	2.0	Smooth	Partial	Even
	2.0	82.0	2.0	Smooth	Partial	Even
	2.5	83.0	2.0	Smooth	Partial	Even
	3.0	83.0	2.0	Smooth	Partial	Even

TABLE 5. EFFECT OF ISABGOL HUSK ADDITION ON MEAN SENSORY SCORES OF *CHAPATIS*

Variety	Level of addition	Colour	Texture	Taste	Flavour	Handfeel
'PBW 138'	0.0	5.2	4.6	4.2	4.8	4.4
	0.5	5.2	5.0	4.8	4.8	4.6
	1.0	5.6	5.4	5.2	4.6	5.0
	1.5	5.8	5.4	5.4	4.4	5.6
	2.0	5.6	5.8	5.6	4.8	5.6
	2.5	5.2	5.6	5.2	4.6	5.4
	3.0	5.0	5.5	5.0	4.0	5.4
	LSD	NS	0.889	NS	NS	0.703
'PBW-222'	0.0	5.0	4.6	4.4	4.6	4.2
	0.5	5.2	5.2	4.8	5.2	4.4
	1.0	5.2	5.4	5.2	4.8	4.8
	1.5	5.4	5.6	5.6	4.6	5.2
	2.0	5.8	5.8	5.2	4.4	5.4
	2.5	5.6	5.4	4.8	4.2	5.6
	3.0	5.4	5.2	4.6	4.2	5.2
	LSD	NS	0.984	1.15	0.947	1.2

LSD - Least significant difference.

NS - Non-significant at p = 0.05

Significant increase in peak viscosity was observed in case of *atta* from 'PBW-138', the values being 362 BU for control and 1375 BU at 3% level of supplementation. The values for *atta* from 'PBW-222' also increased from 457.5 BU for control to 1355 BU at 3% level of supplementation. Rao et al (1985) and Deshpande (1982) also reported an increase in peak viscosity with increase in level of fibre, as the fibre itself imbibed a lot of water.

Characteristics of dough and chapatis : Non-significant decrease was found in mixing time of the dough made from the *atta* of both the varieties. The dough handling became difficult as the dough became sticky with increase in level of isabgol for both the varieties. The results are presented in Table 4. D'Appolonia et al (1978) reported that dough stuck to the sides of the mixer and had to be scraped periodically, till a coherent mass of dough was obtained, when oatmeal bran or oat bran was used in the dough as a source of fibre upto the level of 20% in wheat flour.

Puffing of the *chapatis* was partial with increase in level of isabgol in both the varieties. This could be due to dilution of wheat gluten (Ebeler et al. 1980). Browning of *chapatis* was even in case of both the varieties with increase in level of isabgol addition (Table 4).

Sensory qualities of chapatis: The sensory evaluation on *chapatis* was carried out on a 6-point scale for colour, texture, taste, flavour and handfeel and the results are presented in Table 5. The scores for colour decreased non-significantly for the variety 'PBW-138' whereas, the scores increased non-significantly for the variety 'PBW-222' at all levels of supplementation. The scores for texture increased in both the varieties and varied significantly. The scores for taste also increased non-significantly for 'PBW-222' with increase in level of isabgol addition. The scores for flavour decreased non-significantly at all levels of supplementation for 'PBW-138' whereas scores decreased, significantly for 'PBW-222' (Table 5). The scores for handfeel increased significantly at all levels of supplementation for both the varieties.

Isabgol husk improved the farinograph water absorption, dough development time and stability. Gelatinization temperature decreased and peak viscosity increased. Puffing of *chapatis* was partial at all levels of supplementation. Maximum sensory scores at 2% of supplementation level were recorded for colour, taste, flavour and handfeel. It may be concluded that isabgol husk could be added to *atta* at the level of 2% without altering the qualities of *chapati*.

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RESEARCH NOTE

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Processing Effect on Colour and Vitamins of Green Leafy Vegetables

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The effect of blanching and dehydration on seven green leafy vegetables was studied. Total chlorophylls ranged from 10 to 44% and carotenoids from 42 - 79 µg/g in different vegetables. Though total chlorophylls got reduced during dehydration, the fraction chlorophyll 'a' was more stable than chlorophyll 'b' in all the vegetables except chakota. The retention of carotenoids was found to be 60 to 70% in all the vegetables except honagone, where the retention was 36% during dehydration. The retention of vitamin C during blanching and dehydration was found to be from 32 to 97% and 28 to 74%, respectively. Comparitively, vitamin A retained better to the tune of 36 to 94%. Amongst the vegetables studied, chakota and fenugreek leaves had better retention of colour and vitamins.

Keywords : Green leafy vegetables, Colour, Vitamins, Processing, Nutrient retention, Effect of dehydration, Blanching.

In Indian culinary, green leafy vegetables are one of the most valued components for their colour, flavour and thereapeutic value. Generally, leafy vegetables are valued for their minor nutrients like vitamins and minerals. These serve as good sources of water soluble vitamins such as thiamin, riboflavin, nicotinic acid and vitamin A as well as minerals such as calcium, phosphorus and iron. In our country, though 20-25 varieties are grown, they are considered more as local garden vegetables and the preference is based on the region. The most commonly used leafy vegetables are green and red amaranth, palak, chakota, fenugreek leaves, corriander leaves, green and red honagone, kachi leaves, pudina, drumstick leaves, pumpkin leaves and curry leaves, which contribute to flavour, green colour, minor nutrients as well as medicinal properties. Gopalan et al (1985) reported the nutritional composition of most of the green vegetables. The conventional cooking of these results in the losses of water solubles, minerals and change in colour. Oxalic acid present converts calcium into oxalates, making it unavailable for absorption (Olufemi et al. 1996).

Calcium bioavailabilities of cooked spinach, mustard greens, cowpeas have already been reported (Weaver et al. 1987, 1993, 1997; Olufemi et al. 1996). However, the changes that occur during processing of leafy vegetables with regard to vitamins and colour are less understood. Secondly, because of perishable nature, leafy vegetables are more commonly used immediately after cooking and less attention has been paid towards the preservation of leafy vegetables. In Defence Services, for supplementing vitamins and minerals from natural sources, leafy vegetables-based curry mixes can serve as a part of pack rations. An attempt was made to study the processing effect of few species of leafy vegetables so that suitable recipes can be formulated from these mixes, which would give a balanced supply of minor nutrients.

Seven varieties of green leafy vegetables *viz.* dhantu *(Amaranthus gangeticus),* Khirkhire *(Amaranthus paniculatis),* honagone *(Alternanthera triandra),* chakota *(Atriplex hortensis),* palak *(Beet vulgaris),* kachi *(Solanum nigram)* and fenugreek *(Trigonella fernumgracuem)* of good quality matured leaves available in the local market were procured. The stalks were removed, segregated the clean sound portion, washed in water and drained the water. Leaves were chopped into small pieces and blanched in boiling water (2L), containing sodium bicarbonate (0.1%), magnesium oxide (0.1%) and sodium metabisulphite (0.2%) for 2 min. Blanched vegetable was subjected to dehydration at a temperature of 60°C with a tray load of 2 kg, using Kilburn Model cross air flow drier. Drying for about 4 h brought the moisture level of the dried material to around 4-5%. Separately, but the same method was followed for processing all the vegetables. Except kachi leaves, which required 4 h, the rest of the vegetables took 3^1/2 h for dehydration. Fresh, blanched and dehydrated leafy vegetables were analysed for moisture by air oven method, using Yorco Model air oven, chlorophyll and carotenoid, using hexane-acetone solvent mixture and vitamin C by titrometry, using 2, 6 dichlorophenol indophenol reagent (Ranganna 1986). Vitamin A is expressed after calculation by the conversion factor of 1 mg of β-carotene = 1667 IU vitamin A.

The moisture contents of fresh and blanched vegetables were in the range of 85-90% and dehydrated vegetables had moisture contents ranging from 4 to 5%. The colour of leafy vegetables is mainly due to chlorophyll and carotenoids, but the yellow to orange colour of carotenoid is masked by the green colour. In the leafy vegetables studied, chlorophyll range from 44 to 109 mg% and carotenoids from 42 to 79 µg/g (Table 1). Lower levels of both the colour components were found in fenugreek leaves, but, chlorophyll was the highest in chakota leaves and carotenoids in palak leaves. However, vitamin C was found to be relatively higher in dhantu (72.1 mg%) and

TABLE 1. COLOUR COMPONENTS AND VITAMIN C OF GREEN LEAFY VEGETABLES

Vegetables	Moisture, %	Chlorophyll, mg %	Carotenoids, µg/g	Vitamin C, mg %
Dhantu	87.43	77.87	79.10	72.1
Khirkhire	86.82	71.17	39.90	64.6
Honagone	85.88	109.40	49.98	23.1
Chakota	92.70	59.49	45.40	9.8
Palak	90.10	68.70	78.11	19.2
Kachi	88.54	91.10	69.21	11.5
Fenugreek	85.10	44.40	42.01	15.2

Expressed on as is basis

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khirkhere (64.6 mg%) (both Amaranthus species) and in other vegetables, it ranged from 9.8 to 23.1 mg%. Vanisha and Seshadri (1998) have also reported carotenoids in sixteen green leafy vegetables of western region, in consonance with the present study. *Amaranthus gangeticus* (dhantu) and fenugreek leaves had 226 and 243 µg/g of carotenoids, respectively. In the present study, same species of dhantu and fenugreek had 630 µg/g and 282 µg/g carotenoids, respectively, which may be due to the regional variations.

The processing effect of the colour components of green leafy vegetables is presented in Table 2. For relative expresion, all the values are converted to moisture free basis. In general, total chlorophylls decreased during blanching and dehydration. However, it was found to be increased in chakota, palak and fenugreek and this may be due to the increased losses of soluble solids during blanching. Increased levels of carotenoids in carrots during blanching have also been reported (Baloch et al. 1977; Arya et al. 1979). The highest retention of colour was found in fenugreek (95.6%), followed by palak (89.3%), Khirkhere (88%), chakota (87.3%), dhantu (70.32%), kachi (59.80%) and least in honagone (51.35%) samples on dehydration. Relatively, chlorophyll 'a' had better stability than chlorophyll 'b' in all the samples. Chlorophyll degradation in processed foods and senescent plant tissues has been reviewed by Heaton and

TABLE 2. PROCESSING EFFECT ON COLOUR COMPONENTS AND VITAMINS OF GREEN LEAFY VEGETABLES

Leafy vegetables - Methods	Chlorophyll, mg % Total	'a'	'b'	Carotenoids, µg/g	Vitamin C, mg %	Vitamin A IU
Dhantu						
Fresh	620	461	160	630	573.8	1050
Blanched	616 (99.35)	482 (104.50)	134 (83.75)	619 (98.25)	188.8 (32.90)	1034 (98.48)
Dehydrated	436 (70.32)	385 (83.51)	51 (31.87)	450 (71.42)	164.3 (28.64)	750 (71.43)
Khirkhire						
Fresh	540	408	132	303	490.30	500
Blanched	499 (92.40)	444 (108.80)	55 (41.66)	333 (109.90)	282.00 (57.51)	550 (110)
Dehydrated	475 (87.96)	424 (103.90)	51 (38.63)	200 (66.00)	181.70 (37.05)	333 (66.60)
Honagone						
Fresh	775	449	327	354	163.00	590
Blanched	601 (77.50)	397 (88.40)	206 (62.90)	260 (73.44)	150.00 (92.02)	433 (73.39)
Dehydrated	398 (51.35)	303 (67.40)	95 (29.05)	128 (36.15)	65.00 (39.87)	213 (36.10)
Chakota						
Fresh	815	623	186	622	110	1040
Blanched	940 (115.30)	810 (130)	221 (118.80)	900 (144.60)	97.00 (88.18)	1500 (144.23)
Dehydrated	712 (87.30)	530 (85.07)	182 (97.84)	585 (94.05)	74.00 (67.27)	975 (93.75)
Palak						
Fresh	694	570	124	789	192	1330
Blanched	815 (117.40)	614 (107.70)	203 (163.70)	823 (103.10)	145 (75.52)	1370 (103.00)
Dehydrated	620 (89.33)	508 (89.12)	110 (88.70)	660 (82.70)	122 (63.54)	1100 (82.70)
Kachi						
Fresh	795	480	316	604	100.60	1005
Blanched	605 (76.10)	437 (91.04)	168 (53.16)	628 (103.90)	97.60 (97.60)	1050 (104.48)
Dehydrated	476 (59.80)	379 (78.90)	98 (31.00)	479 (79.30)	48.60 (48.60)	810 (80.59)
Fenugreek						
Fresh	298	203	85	282	95	466
Blanched	396 (132.80)	273 (134.40)	105 (123.50)	301 (106.30)	86 (90.52)	500 (107.29)
Dehydrated	285 (95.60)	225 (110.80)	63 (74.11)	201 (71.20)	71 (74.74)	433 (92.91)

The values in brackets are the percentage retention

Maragoni (1996). The loss of chlorophyll in spinach in terms of conversion into pheophytin was recorded to be about 50% during partial drying to a moisture level of 80% (Mathur et al. 1974). In French beans and peas, the loss of chlorophyll was found to be about 37% during frying (Prabhakar Bhat et al. 1974). However, in the present study, the losses of chlorophyll during dehydration were found to be in the range of 29 to 48% in kachi, dhantu and honagone, while the losses were of low order i.e. from 4.4 to 12.7% in the rest of the vegetables studied.

Retention of total carotenoids on dehydration was highest in chakota (94 %) followed by palak (82.7%), kachi (79.3%), dhantu (71.4%), fenugreek (71.2%) khirkhire (66%) and lowest in honagone (36.1%), indicating that the losses of colour in terms of carotenoids and chlorophyll varied from vegetable to vegetable. The processing effect on vitamins C and A is found to be varying in the vegetables studied (Table 2). The losses of vitamin C during blanching were found to be 2.4 to 9.5% in kachi, honagone and fenugreek, while higher losses were recorded for chakota (12%), palak (24.5%), khirkhire (42.5%) and dhantu (67.1%). Subsequently, on dehydration, the losses were less in fenugreek, chakota and palak recording 74.7, 67.3 and 63.5% retention. Though initial level of vitamin C was lower in fenugreek, chakota and kachi, better retention was recorded for processed samples. On the contrary, dhantu and khirkhire had high levels in fresh samples but the loss was higher during processing. Tapadia et al (1995) reported that losses of vitamin C contents of seven vegetables during processing such as washing, cutting and cooking to be 50-90%. In fenugreek leaves, 30% of vitamin C was retained in cooked vegetable, while in the present study, 74.74% was retained after blanching and dehydration. The loss of vitamin C could be attributed to the increased activities of ascorbic acid oxidizing enzymes, and destruction of vitamin and leaching of vitamin during washing and blanching (Tapadia et al. 1995). Relatively, the loss of vitamin A was lower than vitamin C. The retentions of vitamin A were 36.10 to 93.75%. The retention of vitamin A was highest in chakota, fenugreek, palak recording 93.75%, 92.91%, 82.70%, respectively as compared to other vegetables. All the dehydrated leafy vegetables reconstituted in boiling water within 3 min gave a soft textured product.

In conclusion, the stability of colour and vitamins differs depending on the variety of leafy vegetable processed and proves to be an important parameter for consideration during formulation of instant mixes. Both the vitamins A and C and colour were retained better in chakota and fenugreek leaves compared to other vegetables. This observation along with their medicinal values makes it most promising for inclusion in processed products.

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RESEARCH NOTE

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Verotoxin-Producing *Escherichia coli* (VTEC) in Foods of Animal Origin

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In this study, 109 food samples (mainly meat and meat-products) were screened for *Escherichia coli*. Forty four samples (40.37%) showed *E. coli* and these were tested for verotoxin (VT) production by vero cell assay. Seven (15.90%) isolates were verotoxigenic of which 4 (08, 068, 084, 088) were from buffalo beef and one each was from chicken (086), mutton (non-typable strain) and *kabab* (0159) samples. Variations among the levels of VT production i.e. verotoxin-titre were also recorded.

Keywords : *Escherichia coli,* Verotoxigenic, Meat, Meat-products, Verotoxin-titre.

It has been reported that certain strains of *Escherichia coli* produce a potent cytotoxin named verotoxin (VT), which has a profound and irreversible cytopathic activity on Vero cells (Konowalchuk et al. 1977). For its strikingly similarities both in structure and biological activities to Shiga-toxin produced by *Shigella dysentiriae* type-1, VT is also referred to as Shiga-like toxin (SLT) (O'Brien and La Veck 1983). The VT/SLT-producing *E.coli* (VTEC/SLTEC) have been found to be associated with a broad spectrum of diseases in human, ranging from sub-clinical infections to mild diarrhoea, bloody or non-bloody diarrhoea, haemorrhagic colitis (HC), haemolytic uraemic syndrome, thrombocytopaenic purpura (Karmali 1989) and VT has been proposed as one of the virulence factors produced by these *E. coli* (Karmali et al. 1985; Keenan et al. 1986; Paton and Paton 1998). Epidemiological studies have shown that domestic animals mainly cattle are the principal reservoirs of VTEC (Paton and Paton 1998) and food of animal origin is the major source for human infection (Karmali 1989). The organism can enter human food chain from a number of animal sources mostly by contamination of meat or carcass or plant environment with faeces or intestinal contents before, during or after slaughter. That is why food of animal origin must be regarded as potential vehicle of VTEC (Bulte and Monte negro 1988).

In this study, meat of different animals and meat products were investigated to assess the occurrence of VTEC.

Food samples : One hundred and nine samples representing beef, chicken, mutton, *kabab*, pork and fish were collected from different localities of Bareilly. Meat specimens (approximately 50 g) and *kabab* (meat product) samples were collected in UV sterilized polyethylene sachets. For fish samples, sterile swabs were collected from gills, as well as intestines and kept immediately in sterile test tubes. All the samples were immediately transferred to the laboratory and subjected to detailed bacteriological examination to isolate and identify *E. coli*.

Isolation and identification of E. coli : *E. coli* were isolated from the collected samples following the methods, described by Sojka (1965) and Edwards and Ewing (1972). McConkey broth was used as enrichment medium. A meat or *kabab* specimen (approximately 5 g) was first placed in 20 ml McConkey broth, mixed thoroughly in a blender and then incubated overnight at 37°C. After that, the enriched inoculum was streaked on McConkey agar and eosin-methylene blue (EMB) agar plates and incubated for 24 h at 37°C.

Isolates were confirmed as *E. coli* on the basis of characteristic biochemical reactions as per standard techniques (Edward and Ewing 1972; Cruickshank 1969).

Serotyping of E. coli *isolates :* The isolates on nutrient agar slants were sent to Central Research Institute, National *Salmonella* and *Escherichia* Centre, Kasauli, India, for 'O' serotyping.

Production of enterotoxin : Each of the *E. coli* isolates was inoculated in 5 ml of tryptic soy broth (TSB) (Difco) and incubated overnight at 37°C. From this, 2 ml was inoculated into 250 ml conical flask containing 20 ml TSB and incubated for 18-20 h at 37°C in a shaking water bath. The bacterial cells were pelleted by centrifugation (6000 rpm) at 4°C (Sorrvell RT 6000 Refrigerated Centrifuge) for 30 min and the supernatant was filtered through 0.22 µm membrane filter (Sartorius). The sterility of the cell-free culture supernatant was checked by streaking onto nutrient agar plates and incubating at 37°C for 24 h. The sterile culture supernatant was then stored at –20°C, until use for a few days.

Vero cell cytotoxicity assay : The verocytotoxin assay used, was a modification of the method described by Konowalchuk et al (1977). The Vero cell line was propagated by trypsinization using trypsin-versene. Glasgow minimum essential medium (G-MEM) supplemented with new born calf serum (NBCS) was used for propagation and maintenance of the cell line.

The toxin activity was assayed in 96-well microtiter tissue culture plates (Tarsons). Vero cell culture in each well was seeded and allowed to grow as confluent monolayers for 24-48 h. At the time of assay, the medium was discarded, monolayers were washed twice with Hank's balanced salt solution and 20 µl of each of the culture supernatants was inoculated to the monolayers in triplicates. The medium supplemented with NBCS (200 µl) was then added into each well. In each microtiter plate, culture supernatant of *Shigella*

* *Corresponding Author*

dysenteriae type-1 and 0157 VTEC strain, as positive control and *E. coli* 0111 ab:H-EPEC, as negative control were added to monolayers in triplicates. The microtitre plates were then incubated at 37°C in 5% CO_2 atmosphere and examined daily under an inverted microscope for upto 3 days for characteristic morphological effects i.e., cytopathic effect (CPE). Toxin-preparation inducing CPE in 50% or more cells was recorded as positive and further titrated.

Determination of toxin titre : Serial four-fold dilutions (1:4, 1:16, 1:64, 1:256, 1:1024) of the VT positive toxins were made in G-MEM without any calf serum. From each dilution, 50 µl was added on Vero monolayers in tissue culture plate. In each row, positive and negative controls were kept and observed microscopically, as described above. The reciprocal of the highest dilution inducing CPE in 50% or more cells was recorded as the titre of the toxin.

The isolation rate of *E. coli* varied from 40 to 44% from raw food samples, while only 6 (27.27%) of the *kabab* samples were found to carry *E. coli* (Table 1). Overall, 32(72.73%) serotyped *E. coli* comprising 22 different 'O' serotypes were recognized out of 44 food isolates.

In Vero cell cytotoxicity assay, 7 (15.90%) of the isolates were found to produce verotoxin, of which 4 (O8, O68, O84, O88) were from buffalo beef. One each of the isolates from chicken, mutton and *kabab* were also verotoxic, whereas none of the pork and fish isolates produced VT (Table 1). Microscopically, the VT-affected cells appeared round but shrivelled and many floated free in the medium.

On the basis of initial screening, VTEC isolates were titrated for semi-quantification in vero monolayers. There were quantitative variations among VT titres produced by different *E. coli* strains and these ranged between 16 and 256 with a mean value of 77.7 (Table 1).

TABLE 1. ISOLATION OF VEROTOXIN-PRODUCING *E. COLI* IN DIFFERENT FOOD SAMPLES

Source	No. of sample	No. of *E. coli* isolated, %	No. of verotoxigenic *E. coli*, %	Serotypes of VTEC	Reciprocal of the highest dilution inducing CPE in ≥50 cells (Verotoxin -titre)
Buffalo beef	32	14 (43.75)	4 (28.25)	O8	16
				O68	64
				O84	16
				O88	64
Chicken	27	12 (44.44)	1 (8.33)	O86	64
Mutton	18	8 (44.44)	1 (12.50)	NT*	64
Kabab	22	6 (27.27)	1 (16.66)	O159	256
Pork	5	2 (40.00)	ND**	–	–
Fish	5	2 (40.00)	ND	–	–
Total	109	44 (40.37)	7 (15.90)	–	–

* NT - Non-typeable

** ND - Not-detected

In the present study, of the 7 (15.90%) verotoxic *E. coli* isolates from food, 28.57% (4 of 14) from raw buffalo beef, 8.33% (1 of 12) from chicken, 12.50% (1 of 8) from mutton, 16.66% (1 of 6) from *kabab* were cytotoxic to Vero cells. None of *E. coli* isolates (2 each) from pork and fish was verotoxic.

In other epidemiological studies worldwide on the prevalence of VTEC in beef, detection rate was 36.4% in southern Ontario, Canada (Read et al. 1990), 23% in Seattle, Washington (Samadpour et al. 1994), 17% in London (Willshaw et al. 1993), 14.1% in Netherlands (Heuvelink et al. 1996). Suthienkul et al (1990) isolated VTEC from 9% market beef and 8-28% from fresh beef specimens in a slaughter house in Thailand, whereas Manandhar et al (1997) found only 3% VTEC in raw beef in Australia. Thus, the incidence of VTEC from beef in this study was comparable to those of others.

Detection rates of VTEC from pork have been reported to be 5-18% (Manandhar et al. 1997; Samadpour et al. 1994; Read et al. 1990). Even from fish, VTEC was detected at the rate of 9.67% (Samadpour et al. 1994). On the other hand, no *E. coli* isolate from pork and fish could be detected in VTEC cluster. Analysis of more *E. coli* isolates could give a clearer picture.

Only one VTEC each could be isolated in the present investigation from chicken meat. Such lower prevalence from chicken has been previously reported by Samadpour et al. (1994) and Doyle and Schoeni (1987). But Read et al (1990), Irwin et al (1989), Smith et al (1991) and Manandhar et al (1997) did not get any VTEC from chicken meat. The reason for this is not clear. Furthermore, the VTEC serotype from chicken was O86, which was previously reported to be isolated from water sample (Manandhar et al. 1997).

On the other hand, only one NT-strain of VTEC from mutton was detected. In an earlier survey, Samadpour et al (1994) reported VTEC in 48% lamb samples, whereas Manandhar et al (1997) could not find any VTEC in 29 lamb samples. Because of smaller sample size, it was difficult to arrive at any conclusion on the basis of the available data.

The 4 VTEC strains from buffalo beef in this study were of O8, O68, O84 and O88 serotype. Isolation of serotype O8 and O86 has been reported from beef, too (Read et al. 1990; Heuvelink et al. 1996). The fact that the same serotypes have been detected in faecal samples is indicative of the possibility of cross contamination of the beef during slaughter or subsequent handling of carcass.

Only one VTEC strain (O159) could be isolated from *kabab. Kabab* is generally prepared with ground meat (may be beef, chicken or mutton), which is cooked thoroughly. Thus, isolation of verotoxic *E. coli* from the *kabab* should be regarded as potential threat to the public health. Isolation of *E. coli* from *kabab* has been reported in India (Singh et al. 1996). Isolation of O159 VTEC in beef has been reported from Thailand, too (Suthienkul et al. 1990).

Among the VTEC isolates from raw meats, O8 has been associated with calf diarrhoea and human diseases (Sherwood et al. 1985) and O86 was implicated in HUS in an Italian patient

(Tozzi et al. 1994). But O88 VTEC is yet to be reported in human and/or animal disease.

Among the several VTEC strains, O157 is one of the most important serotypes. But not a single VTEC 0157 could be isolated. Earlier workers, too, could not isolate this serotype from meat and their products (Read et al. 1990; Smith et al. 1991; Willshaw et al. 1993; Samadpour et al. 1994). However, Doyle and Schoeni (1987) reported the isolation of O157 from 1.5-3.7% samples of ground beef, pork, lamb and poultry.

The variations in VT-titres in different *E. coli* strains observed in this study were in agreement with previous reports which decribed the variations in VTEC, isolated from animals and human (Konowalchuk et al. 1997; Karmali et al. 1985; Blanco et al. 1988; Montenegro et al. 1990).

It may be concluded that though India has the largest cattle and buffalo population in the world, the status of VTEC in the country specially in animals and food of animal origin has not been studied. But the emergence of VTEC as important food borne zoonotic pathogen is being highlighted throughout the world due to their association with some life threatening human diseases. So, a closer observation on the organisms is urgently needed to get a clear picture in our country.

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BIOTECHNOLOGY : FOOD FERMENTATION: Volumes I and II, Editors : V.K. Joshi and Ashok Pandey; Education Publishers and Distributors, New Delhi; pp 1372 index XXIV, 1999, Price - not mentioned.

Microbes have been involved in the preparation and processing of foods from time immemorial. While few microbes infect the food and make it unfit for consumption, infection of some other makes it more appetising and nutritious. Tremendous progress in food fermentation has been made though significant contributions from different disciplines such as microbiology, biochemistry, molecular biology, chemical engineering-currently, collectively referred as biotechnology. All these areas are appropriately covered in this book. This book consisting of more than 1400 pages and 32 chapters is presented in two volumes. Individual chapters are written by different authors each demonstrating their expertise in their fields of work. Many of the contributors are from India and few others from overseas. While Volume I covers the contributions from the different disciplines of science to food fermentation, Volume-II deals with different valuable products produced in the food industries for various applications.

Volume-I contains 12 chapters. The title of the first chapter indeed is the title of the book itself. In the absence of a detailed foreword for the book, this serves as the introductory chapter for the book, describing the contents in brief. Microbiological aspects of fermentations of foods covering history, distribution, diversity of microorganisms, factors affecting growth of microorganisms and importance of beneficial and spoilage microorganisms in terms of nutrition and toxicology are presented in detail in chapter 2. Biochemical reactions of fermentations are described with the help of different metabolic pathways in chapter 3. Chapter 4 is an important one covering genetic manipulation of important microorganisms useful to foods such as yeast, fungi, lactic acid bacteria, methods of reproduction, strain modification, cloning of genes, stabilization of transformed microorganisms and their role for the production of commercially valuable products. Importance of fermentation for improving the nutritional value of food products and removal of antinutritional factors is stressed in chapter 5. Major fermented products are briefly presented in this chapter. These products are also described as a separate chapter in Volume-II. Safety of fermentation products is of paramount importantance. Toxins produced by different microorganisms in food/feed make the products unfit for consumption. Commonly found bacterial, fungal and algal toxins in food, their mode of action, control of toxins in food products are separately described in chapter 6.

Commercial success of fermented products depends on the sensory quality of these products. Since the fermentation is a complex process, role of sensory science in developing quality product acceptable to consumers is presented in chapter 7. Different types of fementations, optimization of conditions of fermentation, extractive fermentation for efficient product recovery, contribution of whole viable microbial cell immobilization techniques for improved process development based on fermentation are covered in chapter 8. Development of different fermentor/bioreactor design, problems involved in operation and scale-up of fermentation process with specific examples such as yeast and mushroom biomass production are presented in chapters 9 and 10. Specific requirements for packaging fermented products in different packaging materials and labelling parameters are described in chapter II. Finally, the points one should keep in mind, while developing economically viable fermented products are presented with typical examples such as mushrooms, wine, brandy, ethanol, enzymes, single cell protein etc. in chapter 12.

Volume-II is mainly devoted to various products currently in use in food industries based on fermentation. Each product or groups of products are covered in a separate chapter. Products covered include oriental fermented foods, grape-based fermented products, fruit-based alcoholic beverages, cereal-based alcoholic beverages, fermented milk products, mushrooms, fermented cereal products, fermented fruits and vegetables, fermented meat products, singlecell proteins, mycoprotein, microbial fats, microbial enzymes, Baker's yeast, food additives, ethanol, gums, amino acids, vitamins and organic acids. Industrial success story of production of ethanol by fermentation is appropriately presented as a separate chapter (chapter 28). Food processing industry generates large quantities of biological waste materials. Several of these wastes have great potential for growing microbes. This will reduce not only the waste disposal problem, but also produces a number of valuable microbe-based products. Production of value-added products (chapter 31) and biogas (chapter 32) by fermentation using waste material is also described.

Few topics such as sensory evaluation, packaging and economics of fermented products should have been presented in a single chapter, instead of dealing in separate chapters. This should have reduced the bulk of the book. As in many similarly constructed books in which individual chapters are written by different authors, there is a lot of unnecessary repetition. For example, single cell proteins, different microbial products are described in several places. Occasional typographical errors and mistakes were also crept into the book. Both brief and detailed contents are presented at the beginning of the book. Instead, detailed content of individual chapters should have been presented with the chapters.

In general, the book is well written, with very informative lots of supporting references. I agree with the editors' assertion printed on the back of book that the book is an incomparable source of information for food scientists, biotechnologists, biochemists, fermentation technologists, nutritionists, chemical engineers, microbiologists, toxicologists, UG and PG students.

S.G. BHAT

CENTRAL FOOD TECHNOLOGICAL RESEARCH INSTITUTE, MYSORE-570 013

EVALUATION OF CERTAIN FOOD ADDITIVES: Fifty-first Report of the Joint FAO/WHO Export Committee on Food Additives/ WHO Technical Report Series No. 891. WHO, Geneva pp 168, 2000 Price Swfr 35/- Price in developing Countries Swfr 24.50/-

This report presents the conclusions of the 51st meeting of Joint FAO/WHO Expert Committee convened to evaluate the safety of various food additives, with a view to recommending Acceptable Daily Intake (ADI) for humans. The Expert Committee prepared specifications for the identity and purity of food additives. The first part of the report contains a general discussion on the principles governing the toxicological evaluation of food additives. The assessment of intake of food additives, including critical evaluation of various methods, the limitations, uncertainty and variability associated with these methods for estimating food consumption and intakes of food additives and contaminants have been described. The report provides principles governing the establishment and revision of specifications, comments concerning enzyme preparations derived from genetically modified microorganisms and limits for heavy metals.

The report contains 8 sections covered in 168 pages. Section 1 is the introduction, describing the importance and significance of the joint meeting chaired by Prof. R. Walker held at Geneva between 9 and 18 June 1998.

Section 2 deals with general principles governing the toxicological evaluation of food additives, its intake assessment to set ADI, establishment and revision of specifications.

Section 3 provides the summary of the committee's evaluation of toxicological data on specific food additives including enzyme preparations derived from genetically modified organisms (alpha aceto acetate decraboxylase and maltogenic amylase). Based on the available toxicological data, ADI status was discussed for flavouring agents viz. transanethole, furfural and menthol.

The committee evaluated curcumin, the main colouring component of turmeric oleoresin and suggested a temporary ADI of 0-1 mg/kg body weight per day till 2001, pending the submission of results of a reproductive toxicity study. For use as a colouring agent, riboflavin derived from a fermentation process using a genetically modified strain of *Bacillus subtilis* was evaluated on the basis of its substantial equilence to synthetic riboflavin and established a similar ADI of 0-0.5 mg/kg body weight. According to nutrition experts, a dietary requirement of riboflavin is about 0.6 mg per 1000 calories. The daily safe requirements of this vitamin range from 0.7 to 2.2 mg/day, JECFA fixing an ADI 0.5 mg/kg body weight or 30 mg for 60 kg adult appears to be very high, which is approximately 10 times higher than upper limit of daily requirement of riboflavin. Therefore, this ADI needs to be revised.

The expert committee extended the temporary ADI for mineral oils as glazing agent to 0-1 mg/kg body weight upto 2002, pending the review of reports of the requested studies. A sweetening agent Stevioside is a natural constituent of the plant *Stevia rebaudiana*. It has been reviewed for its safety and toxicity. The committee could not allocate an ADI to Stevioside, because of unavailability of relevant scientific data on the metabolism of Stevioside in humans and suitable *in vivo* genotoxicity studies. The section also contains brief toxicological data on carrageenan-thickening agent, processed *Eucheuma* sea weed and enzymatically hydrolysed sodium caboxymethyl cellulose. Miscellaneous substances viz. gamma-cyclodextrin, glucono-delta-lactone, salts of gluconic acid and polyglycitol syrup were also reviewed for ADI.

In section 4, the committee evaluated the safety and metabolism of 7 groups of flavouring agents which include :

- 13 substances structurally related to menthol
- 39 saturated aliphatic, acyclic secondary alcohols, ketone and related saturated and unsaturated esters
- 42 linear and branched chain, aliphatic unsaturated unconjugated alcohol, aldehydes, acids and related esters
- 23 flavouring agents aliphatic, acyclic, alicyclic tertiary alcohols and related esters
- 8 carvone and structurally related substances
- 21 alpha and beta ionones and structurally related substances
- 22 aliphatic, acyclic and alicyclic alpha diketones and related hydroxyketones

In section 5, the Expert Committee assessed the intake of five food additives viz. benzoates, BHA, BHT, TBHQ and sulphites. This was done in response to a request of the 29th session of the Codex Committee on Food Additives and Contaminants. The committee concluded that the mean intakes of the above additives estimated from model diets or based on national levels of use, or from analysis of individual dietary records in different countries, suggest that the current mean intakes are below ADI. Although some methods for estimating intake, notably those based on maximum limits and range of foods specified in the draft General Standard for additives showed that consumption may exceed ADI.

Sections 6, 7 and 8 are concerned with revision of certain specifications, future work and recommendations, respectively. Annexed to the report are tables summarizing the committee's recommendations for ADIs of the food additives considered, changes in the status of specifications for these substances, and further toxicological studies and other information required or desired. Last Annexure depicts Food Categorization System consisting of 16 groups.

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HYDERABAD-500 007

AFST (I) News

35th Annual General Body Meeting of AFST (I)

The 35th Annual General Body Meeting of the Association was held on November 24, 2000 at the IFTTC Auditorium, CFTRI Campus, Mysore. The meeting was attended by 110 members.

Dr. M. Mahadeviah, President, AFST (I), welcomed the members. He thanked the members, colleagues and office staff, who worked hard in making ICFOST-2000 a grand success. Mr. R. Naresh, Hon. Exec. Secretary of AFST (I), presented the minutes of the previous Annual General Body Meeting held on December 1999 at IFTTC Auditorium, CFTRI Campus, Mysore. The General Body unanimously approved the minutes. Dr. U.J.S. Prasada Rao, Treasurer, presented the Finance Report, which was unanimously approved by the General Body. It was unanimously decided to continue Sri. A.K. Krishnamurthy as the auditor. There was some discussion regarding amendments to the constitution of the Association to correct certain anomalies. Members expressed their views in this regard.

Dr. M. Mahadeviah then requested Dr. S.R. Padwal Desai, the President-designate of AFST (I) to take over charge as the President for the year 2000-2001. Dr. Padwal Desai in his acceptance speech congratulated the previous office bearers for the excellent work done during their tenure. He sought the cooperation from members during his tenure.

Dr. V. Prakash, Director, CFTRI, Mysore suggested that AFST (I) should set agenda for the next 10 years to make the Association a force to reckon in policy matters regarding food and allied areas. He extended CFTRI's cooperation to the Association in fulfilling its commitments and vision for the new millennium.

Dr. K.S. Manja, Director, Defence Food Research Laboratory, Mysore offered best wishes and extended his support for the activities of the Association.

Mr. R. Naresh, Hon. Exec. Secretary thanked the office bearers for their cooperation and help in conducting ICFOST-2000 successfully.

Following is the list of members of the Central Executive Committee (2000-2001) :

President	Dr. S.R. Padwal Desai
President-Designate	Dr. S.C. Basappa
Imm. Past President	Dr. M. Mahadeviah
Vice-Presidents	Dr. M.S. Prasad (H.Q)
	Dr. A.K. Guha (Kolkata)
	Mr. B. Raghuramaiah (Chennai)
	Dr. A.S. Gholap (Mumbai)
	Dr. P.G.Adsule (Pune)
Hon. Exec. Secretary	Mr. Akmal Pasha
Jt. Secretary	Dr. U.J.S. Prasada Rao
Imm. Past Secretary	R. Naresh
Hon. Treasurer	Dr. Y.N. Vijayashankar
Editor-in-Chief, JFST	Dr. B.R. Lokesh
Chief Editor, IFI	Mr. G.C.P. Rangarao

AFST (I) Award Winners for 1999

Prof. V. Subrahmanyan Industrial Achievement Award

Sri B.R. Bedekar, Food Technologist, Pune

Fellows AFST (I)

The following five eminent Food Scientists and Technologists were conferred the title 'FELLOW' AFST at the AGBM.

Prof. M.A. Rao, Cornell University, Geneva, U.S.A.,

Mr. A. Ramesh, Central Food Technological Research Institute, Mysore, India

Prof. G.S. Chauhan, GB pant University of Agriculture and Technology, Pantnagar, India

Mr. B. Raghuramaiah, Food Ingredient Specialties Pvt. Ltd., Chennai, India

Dr. R.N. Tharanathan, Central Food Technological Research Institute, Mysore, India

Laljee Godhoo Smarak Nidhi Award

Dr. Iddya Karunasagar, University of Agricultural Sciences, College of Fisheries, Mangalore

Subhash Bhatnagar Memorial Award

Mr. G. Mohan Kumar, Fruitein Exports, Mysore

Young Scientist Award

Dr. S.K. Otta, College of Fisheries, Mangalore

Best Student Award

Ms. P. Preeti, Smith Kline Beecham Consumer Healthcare, New Delhi

Best Paper Award

'Application of Sodium Erythorbate in Controlling Browning of Diced Potatoes' by Hema Sarath Gopalan, M.D. Pendharkar, G. Subbulakshmi and S.R. Padwal Desai, published in the 'Journal of Food Science and Technology' Vol. 36, No. 3, May-June, 1999, pp.226-234

Best Feature Article Award

'Asceptic Processing and Packaging: Promising Packaging Technique for Food Processing Industries' by G. Tewari and D.S. Jayas, Indian Food Industry, Vol. 18, No. 1, January-February 1999, pp.23-33.

IMPORTANT ANNOUNCEMENT

The last date for receiving nominations for the various AFST (I) awards for the year 2000, has been extended to **30, April 2001**

Secretary

Association of Food Scientists and Technologists (India)
Journal of Food Science and Technology

Instructions to Authors

Journal of Food Science and Technology, devoted to original Research and Development contributions in all branches of Science, Technology and Engineering of Foods and Food Products, is a bimonthly publication of Association of Food Scientists and Technologists (India), Mysore. The journal publishes the following types of papers.

1. Research papers with a maximum length of 16 manuscript pages, including figures, tables and references
2. Research notes which are limited to a maximum length of 8 manuscript pages, all inclusive.
3. Reviews on specific topics of higher utility and current trends. The reviews should be of about 45-50 manuscript pages. Mini reviews of about 20-25 manuscript pages are also considered.

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The manuscript submitted to *Journal of Food Science and Technology* will be processed with clear understanding; that the work presented in the paper has not been published before; that it is not under consideration for publication elsewhere; that its submission has been approved by all co-authors and by the responsible authorities of the host institute where the work was carried out; and that the manuscript will not be published elsewhere in any form without the consent of the copyright holders.

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Journal of Food Science and Technology

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Manucripts

The manuscripts of the research paper should be arranged in the following order.

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Each paper should be provided with an abstract of up to **250 words** reporting concisely the objectives, approach and the principal findings of the work and in.the form acceptable to abstracting agency. This should be followed by at least **6 keywords** and this should include all the main topics discussed in the paper.

Text

The text should start on a new page with introduction (without heading), Methods and Materials, Results, Discussion, Acknowledgement (if any) and References. Results and Discussion may be combined. Research notes will not have any sub headings except for Abstract and References.

Materials and Methods must give sufficient details for the work to be repeated. Methods of sampling, number of replications and relevant statistical analysis of data should be indicated. Any hazard must be mentioned and the relevant safety precautions or reference to safety procedures must be made. The use of proprietary names should be avoided. The chemicals are to be referred by names and not by formula in the text. All vernacular names *e.g., pulav, puree, chapatti etc.* must be underlined to indicate

those to be in Italic types and briefly described when appearing for the first time. All varietal names such as IR 150, Pb-58 must be put in single inverted commas. Tables, numbered consecutively with Arabic numerals are to be typed on separate sheet and placed after reference section. No verticle lines should be used and the table should not have more than 12 columns. No results should be indicated by using ND (not detected, while absence of data by the sign,'-'.

References should be cited at the appropriate points in the text by giving authors' names and year in bracket i.e. (Smith 1990; Smith and Gibbons 1990; smith et al. 1992) as per Harvard System. A list of references, in alphabetical order, should appear at the end of Results and Discussion section. In case of single author reference, the alphabetical arrangement should be by data, while it should be by co-author when the reference with one co-author is cited. The alphabetical arrangement should be by data in case of the reference with more than one co-authors. Abbreviations such as et al., ibid., idem must be avoided. The titles of all scientific periodicals should be abbreviated as in Chemical Abstracts, Biological Abstracts, Annual BIOSIS, List of Serials and Serial Sources for the BIOSIS Data Base. If the journal is not included in these sources, then the name of the journal is to be given in full. Unpublished data or private communications should not appear in the list, but can be indicated in the text. The examples of the layout of typical references are as under :

Published papers/notes/reviews

Tairu AO, Omotosu RA, Bamiro FO (1991) Studies on oxidative stability of crude and processed yellow nutsedge tuber and almond seed oil. J Food Sci Technol 28:8-11

Books/approved methods

a) Hacking AJ (1986) Economic Aspects of Biotechnology, Cambridge University Press, Cambridge.

b) AOAC (1984) Official Methods of Analysis, 14th edn. Association of Official Analytical Chemists, Washington, DC

Chapter in edited books/book series/papers in symposium proceedings/souvenir

a) Kurtzman CP, Phaff HJ, Meyer SA (1983) Nucleic acid relatedness among yeasts. In: Yeast Genetics, Fundamental and Applied Aspects. Spencer JFT, Spencer DM, Smith ARW (eds) Springer-Verlag, New York, pp 139-166

b) Gross E (1975) Subtilin and nisin: The chemistry and biology of peptides and α-β saturated amino acids. In: Peptides, Chemistry, Structure and Biology: Walter R. Merenhoper J (eds) Proceedings of the Fourth American Peptide Symposium, Ann Arbor, Michigan, USA. pp 31-42

c) Bhalerao SD, Mulmulay GV, Potty VH (1989) Effluent management in food industry. In: Souvenir, National Symposium on Impact of Pollution in and from Food Industries and its Management. Association of Food Scientists and Technologists (India), Mysore. pp 1-31

Reports by specified authors/Institutions

a) Andress EL, Kuhn GD (1983) Critical Review of Home Preservation Literature and Current Research, Co-operative Agreement No. 12-05-300-553. USDA and Pennsylvania State University, Pennsylvania

b) USDA (1977) Home Canning of Fruits and Vegetables. Home and Garden Bulletin 18, United States Department of Agriculture, Washington, DC

Patents

Schmidt GR, Means WJ (1986) Process of preparing algin/calcium gel-structured meat products. US Patent 4 603 054

Thesis

Ramesh MV (1989) Production of heat stable alpha-amylase. Ph.D. Thesis, University of Mysore, Mysore, India.

Papers presented at symposia

Stevens KA, Klapes NA, Sheldon BW, Klaenhammer TR (1991) Anti-microbial action of nisin against *Salmonella typhimurium* lipo-polysaccharide mutants. Paper presented at 91st American Society for Microbiology, Annual Meeting, Dallas, Texas, USA, 5-9 May

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